(Unclassified)

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THE DISTRIBUTION OF BLOOD FLOW IN HUMAN SKIN

FINAL REPORT

by

J. M. Crismon, M.D. Responsible Investigator

and

Timothy O. Clarke Research Assistant

November 1971

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No DA - 49 - 193 MD-2311

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Summary

Studies carried out under this contract have been directed toward (a) an examination of the nature of blood flow distribution within the skin and (b) a study of participation of superficial dermal capillaries in major changes of skin blood flow rate. This report consists of four Parts. Three Appendixes contain technical details of equipment construction and development. Part 1 contains a statement of objectives and a section on background. Part 2 is a summary of preliminary work including initial hypotheses and development of methods. Results of experiments testing the feasibility of using clearance rate of radioactive isotopes to measure effective skin blood flow showed that the method was too slow to display rapid changes in blood flow and potentially dangerous for repeated use in the same subject. Existing methods of measuring total forearm blood flow were not suitable for our needs. The development of temperature-stable forearm blood flow gauges produced two potentially useful designs. A small, light-weight gauge that was based on changes of capacitance with changes of arm volume was little affected by temperature, but solution of cable and calibration problems would have required an additional year's work. The device finally developed was a mercury-in-silastic rubber gauge modified from the original design by Whitney (21). The improved gauge can be calibrated electrically *in situ* by means of part of the control circuit. The relationship of change in arm volume, after venous occlusion, to voltage output is independent of temperature.

Part 2 also describes preliminary work with helium transfer through the skin as an index of effective skin blood flow. Helium was collected from plastic capsules cemented to forearm skin. When the subject breathed a mixture of 80% helium and 20% oxygen, a collection period of 10 minutes yielded sufficient helium from about 20 cm^2 of skin to permit accurate measurement on 1 ml samples by gas chromatography. Data are presented on simultaneous measurements of skin temperature, total forearm blood flow, sweat rate and helium flux through the skin under various conditions. Effects of heat, cold, posture and exercise, and shunting of blood flow through arteriovenous anastomoses are described.

Part 3 presents the development of equipment and procedures for continuous analysis of helium at a sensitivity about six orders of magnitude greater than that of the gas chromatograph. The high sensitivity was made possible by sweeping a stream of air over about 13 cm^2 of skin and delivering about 1/300 of the 0.5 ml/sec flow to a mass spectrometer-type helium leak tester. Additional studies were carried out on effects of upright posture on skin blood flow distribution. The change from reclining to upright posture was simulated by application of

short periods of lower-body negative pressure (LBNP) to reclining subjects. During the redistributions of peripheral resistance evoked by the decreased cardiac output during LBNP, forearm blood flow decreased. The small changes in skin temperature and the slight decreases in helium leak rate suggested that the resistance vessels of the skin were engaged to a relatively small extent in the reflex vasoconstriction.

The relationships of minute volume and distribution of skin blood flow to processes of heat loss were studied in detail. During general body heating of resting subjects, helium leak rate increased in proportion to rising skin temperature. Direct participation of sweat gland activity in the increased rates of helium transfer was tested by adding 7% CO_2 to the breathing mixture. Inhalation of carbon dioxide evokes sweating when core and skin temperatures are kept below sweating threshold. Although sweating was produced in small amounts by CO_2 inhalation, the changes in forearm blood flow and helium leak rate were inconstant if skin temperatures were maintained at or below 35.5°C. When ion transfer of atropine into the skin of one forearm was used to block sweat secretion in heated subjects, the blood flow of the atropinized arm rose above that in the control arm. Helium leak rates were also higher in the atropinized arm than in the control arm in spite of the absence of sweating in the blocked arm and vigorous sweating in the control arm. Direct heating of one arm of a subject exposed to comfortably cool ambient temperature produced extremely high helium leak rates and large increases of forearm blood flow. Isoproterenol administered by ion transfer to one arm produced relaxation of resistance vessels in the treated skin, but there was no evidence that precapillary sphincters of superficial dermal capillaries responded to the vasodilator. The differences in helium leak rate and forearm blood flow between the treated arm and the control arm were similar in direction to those produced by local heating but much smaller in magnitude.

Part 4 contains a general discussion of results, including comparison of our data on the relation of helium leak rate to skin temperature and theoretical calculations of skin blood flow required for maintaining core and skin temperatures constant. Our data are also compared to findings of others. Conclusions based upon our findings are: 1. The rate of leakage of helium through skin is directly proportional to the capillary area available for diffusion and inversely proportional to the distance from the diffusing surface to the skin surface. When the number of open superficial capillaries increases, the local perfusion rate increases. Therefore the helium leak rate may be used as an index of blood flow distribution in the skin. 2. If some of the skin blood flow is shunted through channels other than the superficial capillaries,

the rate of helium leakage is small relative to the total skin blood flow. 3. The distribution of blood flow in forearm skin is very little affected by barostatic reflexes such as those involved in redistribution of peripheral resistance in response to changing from reclining to upright posture. 4. Circulation of increased amounts of blood in vessels supplying the coiled portion of sweat gland ducts provides a functional blood shunt during responses to general body heating. Little decrease of resistance to flow occurs in the superficial capillary bed when the skin is cooled by evaporation of sweat. 5. Interference with the evaporation of sweat in subjects responding to high ambient temperature or direct application of heat to the skin increases the number of open capillaries as indicated by increased helium leak rate, erythema, and direct counts of visible capillary loops. 6. The precapillary sphincters of the most superficial distribution in the skin do not dilate in response to any of the various changes of nerve activity involved in the heat-loss response. They are controlled by autoregulation in response to local heat or to some feature of the metabolic response to a change in heat.

Recommendations included in the report are: 1. Studies should be carried out to determine the possible usefulness of measurements of helium leak rate to plastic surgeons interested in the rate of revascularization of skin grafts. 2. Studies should be carried out to determine the possible role of dilatation of superficial skin vessels in heat exhaustion and heat stroke. Both effective blood volume and lowered total peripheral resistance may be involved. 3. Studies should be carried out to determine how circulatory stress is related to the rate of acclimatization to heat. Heat acclimatization might occur more rapidly and more consistently in subjects in whom the heat stress included judiciously limited increases in skin temperature.

Foreword

In conducting the research described in this report, the investigators adhered to the principles embodied in the Declaration of Helsinki. The authors wish to acknowledge with deep gratitude the helpful cooperation of those who served as subjects in the experiments.

Material used in preparing figures 4-1 and 4-3 has been included in this report with the author's permission.

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PART 1

Object and Background

Object

The studies carried out under this contract have been directed toward: (a) an examination of the nature of blood flow distribution within the skin under widely differing rates of perfusion, and (b) a study of participation of superficial dermal capillaries in major changes of skin blood flow.

Background

Blood flow in the entire skin of an adult man may vary from about 200 ml per minute to more than 2,000 ml per minute. The minimum flow rates are ample for the metabolic needs of the cellular elements, which make up about 10 per cent of the bulk of the organ. The chief functional role of the wide range of blood flow is concerned with regulation of body temperature. Changes in core temperature and in skin temperature participate as stimuli reaching the spinal cord and brain stem, where integrated changes in adrenergic efferent outflow to cutaneous vascular smooth muscle modify both the resistance to blood flow and the capacity of veins.

Regional differences in anatomical arrangement and processes of flow regulation characterize skin of two general types. Blood flow through the palmar and plantar skin of digits and parts of the nose and ears rises to maximal levels after sympathetic denervation or blockade of regional cutaneous nerves with local anesthetics. In the skin of the dorsal parts of the digits, hands and feet, and the skin of the arms, legs and trunk, sympathetic denervation or blockade increases blood flow by about one third of the maximum levels reached during vigorous sweating in heat exposure. Both sweating and maximum vasodilatation of arm, leg and trunk skin thus appear to require active nerve traffic (1).

The plantar and palmar skin of the feet and hands and the respective digits is richly supplied with arteriovenous anastomoses (2, 3). During the decreased activity in adrenergic sympathetic nerves associated with the cutaneous vascular response to general body heating, the A-V anastomoses dilate. They are believed to carry as much as 95 per cent of the increased blood flow. Blood flow estimations by means of digital calorimetry agree reasonably well with simultaneous measurements by venous occlusion

plethysmography (4). Since the A-V anastomoses are relatively thick-walled structures, they conduct blood from arterioles to venules without providing any opportunity for exchange of water or solutes between the circulating blood and the tissues. Heat exchange appears not to be affected: thermal conductivity of skin is directly proportional to the blood content of tissues (5).

Arterio-venous anastomoses have not been identified in the skin of the arms, legs and trunk. Thus the entire volume of blood that enters the skin vasculature of these regions must pass through some capillary bed before entering the venular plexuses and veins. During exposure to heat, these non-acral skin areas undergo initial vasodilatation owing to decreased frequency of firing of adrenergic sympathetic nerves, having their richest distribution to the smooth muscle of arterioles, small arteries and venules. The degree of vasodilatation achieved passively accounts for about one third of the maximal flow that can be measured at the peak of sweating. The sweating response during heat exposure requires increased frequency of discharge over cholinergic sympathetic fibers that have their terminations at the coiled portion of the eccrine sweat glands. No cholinergic nerve fibers have been found to innervate blood vessels in the skin.

Fox et al proposed that the activated sweat glands produce an enzyme capable of splitting the polypeptide bradykinin from alpha-2-globulin. They suggested that the vasodilatation responsible for the full development of increased blood flow in non-acral skin was attributable to bradykinin (6). In support of this suggestion was their finding of strong bradykinin-like activity in samples of sweat and in fluid recovered from subcutaneous depots of saline injected into volunteer subjects exposed to general body heating. Others (7, 8) have denied that bradykinin is the agent responsible for the major part of the vasodilator response.

Anatomical studies of the distribution of blood vessels in the skin have added to the mounting evidence against the bradykinin hypothesis (9). The blood supply to the eccrine sweat glands comes from arterioles that branch from the arterial plexus at the dermal-subcutaneous junction. The coiled portion of the gland is invested with a net of capillaries and metarterioles that give off branches that wind around the straight portion of the gland and the duct. At the level of the sub-papillary arteriolar plexus, micro-vascular connections occur between the peritubular capillaries

and the beginnings of the capillary loops that extend into the dermal pegs. It is thus conceivable that bradykinin elaborated in the vicinity of the coiled portion of the sweat glands might be carried to the precapillary sphincters of the most superficial microvessels of the skin. It is more difficult to see how bradykinin could reach the arterioles of the dermal and sub-papillary plexuses.

Excess acetylcholine, diffusing from cholinergic sympathetic nerve endings at the sweat gland, seems unlikely as the vasodilator agent on two grounds. Cholinesterases in both blood and tissue degrade acetylcholine in most other organs innervated by cholinergic nerves. Atropine administered by intra-arterial injection (10) or introduced into the skin by ion transfer effectively blocks sweat secretion during general body heating, but cutaneous vasodilatation is only moderately delayed in the atropinized area.

Although no arterio venous shunts of the type found in acral skin have been found in the non-acral skin, it is possible that a form of functional shunting could result from the vasodilatation incident to activation of eccrine sweat glands. The greatest drop in cutaneous arteriolar resistance under these conditions would occur at the level of the coiled portion of the sweat glands, with some dilatation extending to the vessels that accompany the straight portion of the duct toward the skin surface. Since the number of sweat glands activated by general body heating has been reported to be about 230 per cm^2 in forearm skin (12), an appreciable amount of additional blood could be made available to fill the most superficial skin capillaries. Acetyl-beta-methylcholine (Mecholyl) administered by ion transfer activates sweat glands, but it also relaxes vascular smooth muscle. Sweating under this form of stimulation is accompanied by a marked increase in the number of open superficial capillaries, and total skin blood flow is increased. When sweating is produced by general body heating, there is an increase in skin blood flow, but the number of open superficial capillaries is said to be not increased (13).

Little is known about autoregulation of human skin blood vessels. Reactive hyperemia has been studied by local application of pressure sufficient to empty superficial blood vessels (14). The duration of redness following removal of compression was of the same order as the duration of increased blood flow measured in entire extremities after release of arterial occlusion. No useful data are available from these studies to permit separate assessment of metabolic and myogenic mechanisms.

As a first assumption based upon studies in animals, it may be supposed that both myogenic and metabolic autoregulation occur in human skin. Apart from evoked sweat gland activity, the chief mechanism likely to modify metabolic activity in the skin is heat. The precapillary sphincters are the most accessible parts of the vascular smooth muscle to metabolic elevation of excitation threshold. As increased numbers of capillaries fill following relaxation of their sphincters, less total resistance to flow from arterioles is offered. Local arteriolar pressure falls, and the first stage of the myogenic part of autoregulation begins with arteriolar smooth muscle response to decreased wall tension. This myogenic relaxation of arterioles has two effects: it initiates the retrograde involvement of small, then large, arteries, and it transfers the major site of vascular resistance to flow on to the capillary bed beyond. Minute volume blood flow through the capillary bed increases, and both increased diffusion of solutes and outward filtration of plasma ultrafiltrate occur (15, 16). The above sequence of events has been documented by studies on isolated organs and isolated body parts of experimental animals.

Four lines of evidence summarized by Shepherd (17) support the contention that the increased blood flow measured plethysmographically in the forearms of heated subjects is confined to skin. Although the procedures used may be adequate as means of estimating the total increment of blood flow in skin, they provide no information about the distribution of blood flow via "nutrient" capillaries and that through shunts affording no blood-tissue exchange other than that of heat.

Resting men sweating in a hot, relatively dry environment are usually able to maintain a skin temperature below that of the ambient air (18). The total skin blood flow is increased, but cutaneous edema is not conspicuous, and the number of visible superficial capillaries is not remarkably increased (13). If a similar increment of skin temperature and skin blood flow is evoked by local heating of the forearm in men exposed to a comfortable environmental temperature (22 - 25°C), there is local redness of the heated skin, a temporary edema is produced and the number of visible superficial capillaries is increased (19). The two examples described may represent modulation of the distribution of increased blood flow by autoregulation occurring in the most superficial capillaries. Cooling of the skin by the evaporation of sweat may promote closure of precapillary sphincters in the superficial distribution. Direct heating

of the skin may increase local metabolic rate, decrease the ability of the precapillary sphincters to respond to constrictor influences and increase minute volume flow through a larger number of open superficial capillaries.

PART 2

Summary of Preliminary Work Under this Contract

Initial hypotheses and methods

If all blood flowing from arteries to veins through non-acral skin passes by way of "nutrient" capillaries, the transfer rate of any exchangeable material would vary as some function of the change in skin blood flow, the capillary permeability and the capillary area available for exchange. Simultaneous measurement of total blood flow and effective capillary blood flow in the skin with a suitable tracer should yield information about the presence or absence of non-exchange shunts. The additional necessary condition is that the changes of blood flow rate evoked during the procedure be confined to skin.

The forearm and the calf of the leg provide skin areas that are typical of non-acral skin (10). Measurement of total blood flow is possible by venous occlusion plethysmography, and skin blood flow can be changed, in resting subjects, by local or general body heating or cooling with little or no change in muscle blood flow (20).

For total blood flow measurements, the water-filled plethysmograph provides excellent local temperature control, but does not permit convenient access to the same skin areas for measurement of effective capillary blood flow. The mercury-in-rubber strain gauge plethysmograph described by Whitney (21) was selected because it permitted the application of sensors for skin temperature, sweat rate, and effective capillary blood flow simultaneously in skin of the same forearm. (For details of equipment and procedure see Appendix I.)

The method first described by Kety (22) appeared to offer promise as a means of assessing effective capillary blood flow. Kety used 22-Na as sodium chloride to measure the rate of removal of the gamma emitter by the circulating blood. In our procedure we have preferred ^{131}I as sodium iodide for the tracer because of its larger diffusion coefficient and its short half-life, 8.5 days. The subjects could be protected by blocking the thyroid with orally administered Lugol's solution, 8 - 10 drops prior to the experiment.

No method involving injection of even extremely small volumes of material may be considered entirely free of

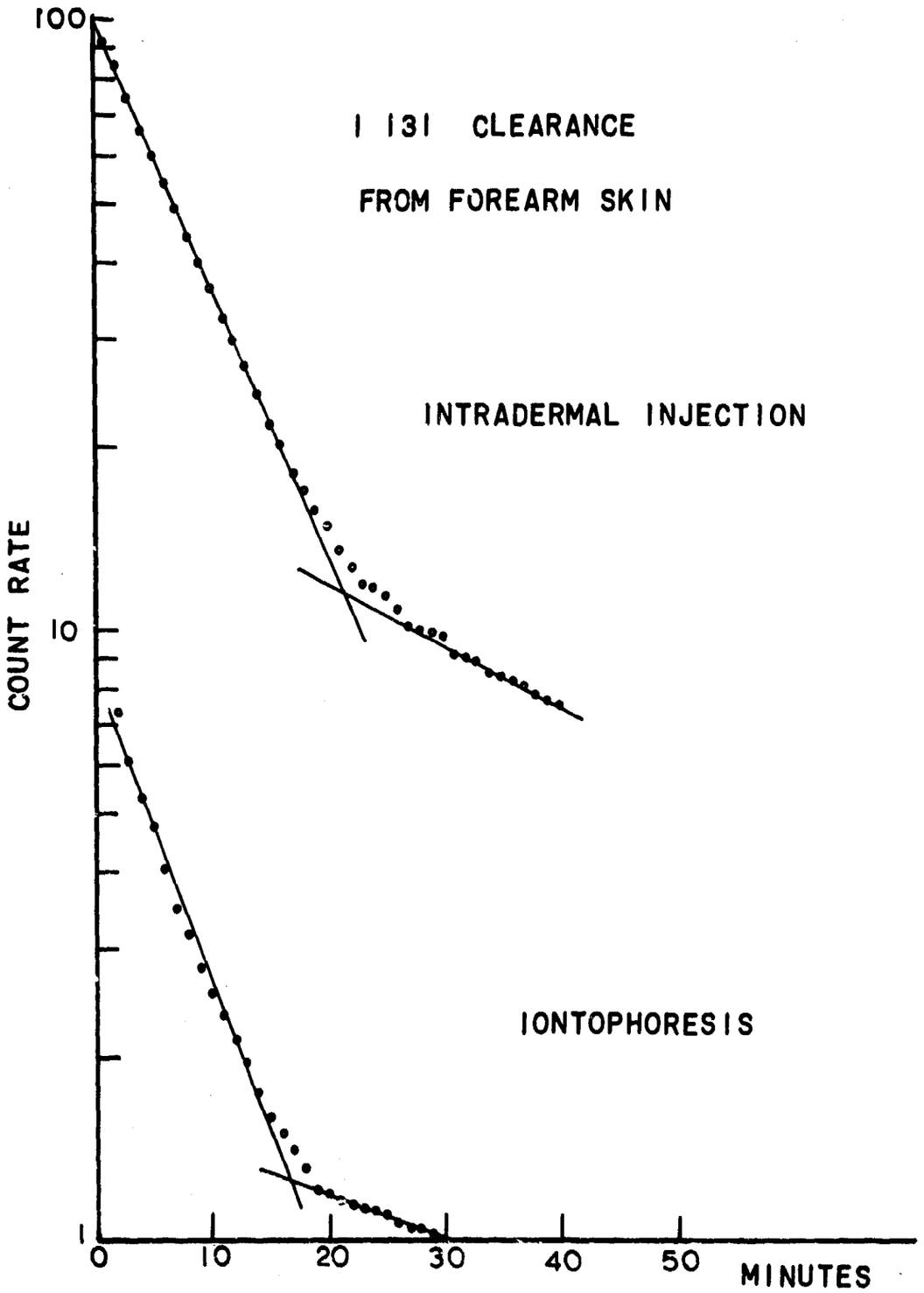

Figure 2-1 131-I clearance from forearm skin.

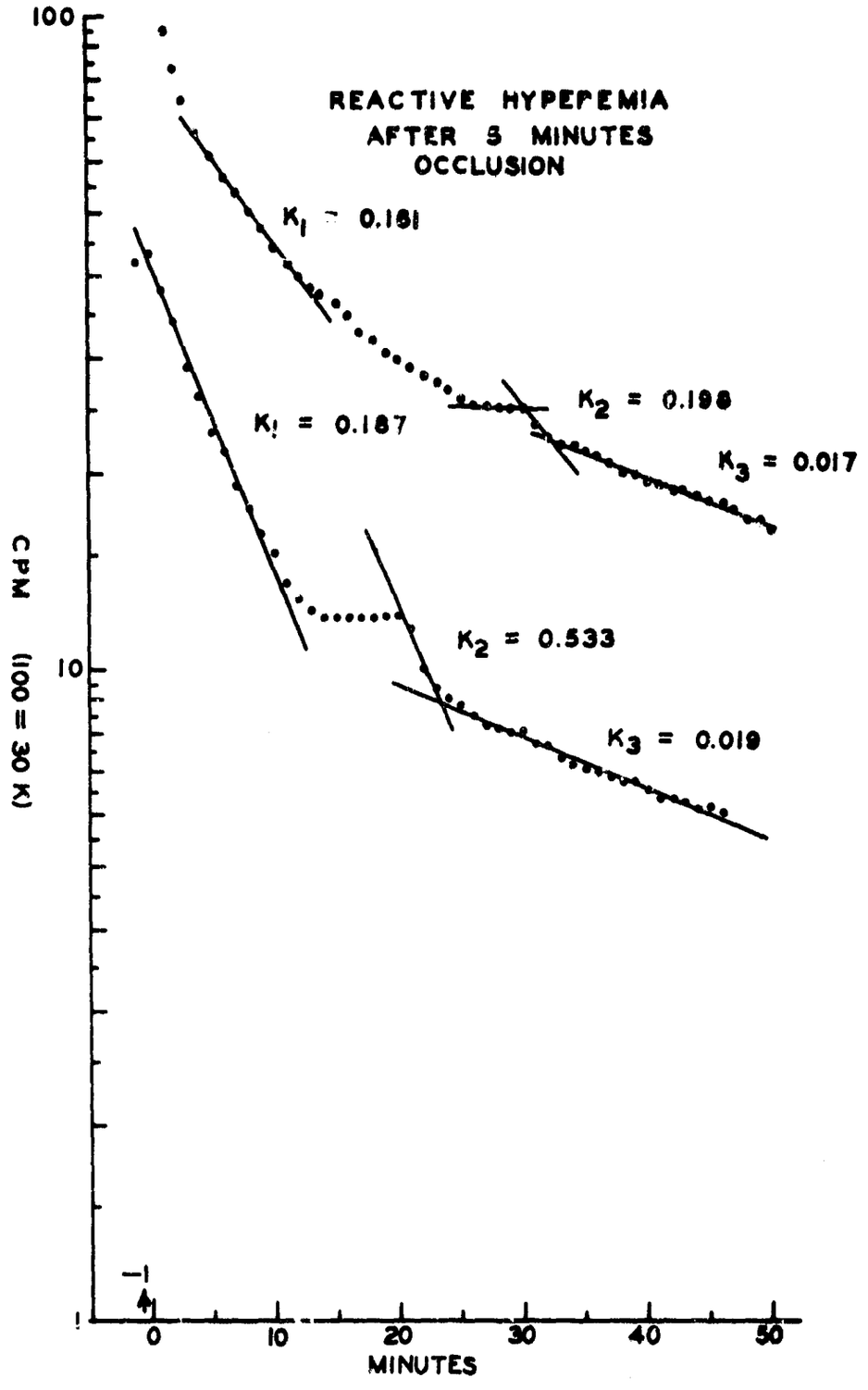

Figure 2-2 Reactive hyperemia after 5 minutes occlusion.

reactive errors. The introduction of the finest needle, of itself, gives rise to local vascular responses of varying duration. The distortion of interstitial tissues by finite volumes of injected solution also creates mechanical alteration of flow in neighboring capillaries of unknown nature, magnitude and duration.

It was decided to use ion transfer (iontophoresis) as the means of introducing 131-I into the skin. Figure 2-1 shows representative plots comparing removal rates of tracer after intradermal injection and after introduction of tracer by ion transfer in the same subject. Details of the ion transfer procedure and the measurement of removal rate are given in the 1962 report to the U. S. Army Medical Research and Development Command (23).

Critical evaluation of initial methods

The methods described above were used to study the effects of reactive hyperemia, general body heating, and the action of topically applied rubifacient substances on total forearm blood flow and on clearance of 131-I from the skin.

The increased blood flow of reactive hyperemia was reasonably reflected by the steeper slopes during the first 18 to 20 minutes after introduction of isotope, (Figure 2-2, upper curve). If release of arterial occlusion was delayed until 20 - 25 minutes after the introduction of isotope (Figure 2-2, lower curve), the rate of removal was little changed; factors other than blood flow appeared to dominate the clearance of isotope from skin 25 to 40 minutes after its introduction.

N-hexyl nicotinate, an active rubifacient applied to the skin by inunction of a 2 per cent cream, increased both total forearm blood flow and flow through superficial capillaries as shown by simultaneous measurements with 131-I clearance and plethysmography. The increased total forearm blood flow was entirely attributable to increased blood flow in the skin (24). Comparison of relative increases in total forearm flow and increased rates of clearance of isotope showed the fractional increase of total forearm flow to be greater than the fractional increase of rate of removal of 131-I. This observation was interpreted to mean that the effective or "nutrient" skin blood flow through the region sampled by the clearance procedure was smaller than the total blood flow per unit area of skin.

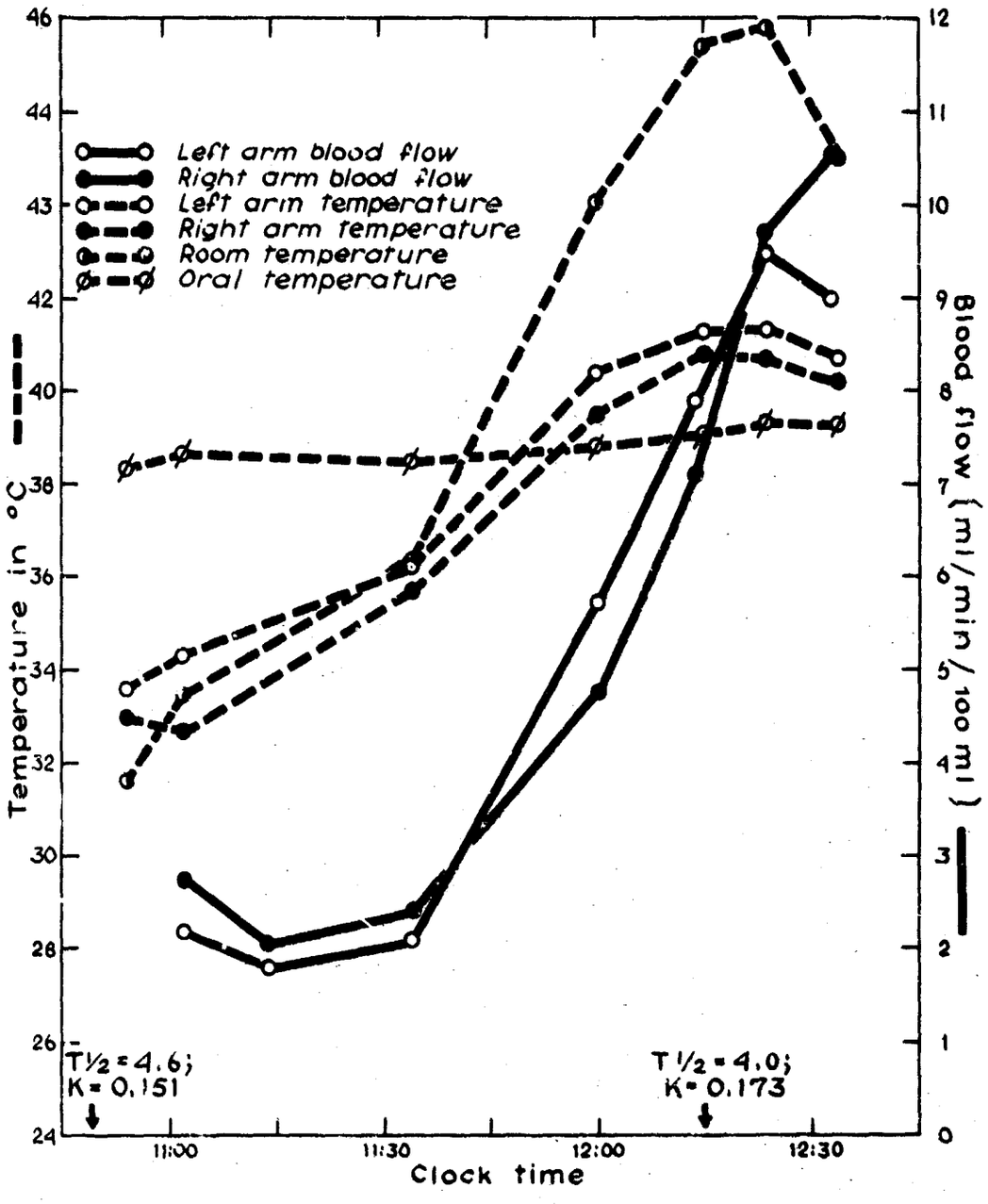

Figure 2-3 Time course of changes in skin temperature and forearm blood flows during general body heating, with two measurements of 131-I clearance (times shown by arrows on the abcissa).

When studies were made on men during general body heating, neither of the two methods for assessing skin blood flow was satisfactory. The temperature compensation of the mercury-in-rubber strain gauges, used for total forearm blood flow measurement, was completely inadequate to maintain calibration over the range of 25 to 48°C spanned by the ambient temperature during the course of a single experiment. The period of exposure to heat lasted 90 to 120 minutes in each experiment, and the duration required for 131-I clearance measurement was a minimum of 30 minutes. By means of the undesirable practice of subjecting each volunteer to a double dose of radiation in each experiment it was possible to test effective skin blood flow at the beginning and at the end of the heating period (23). Figure 2-3 shows the data from such an experiment. The two arrows mark the time of the mid-point of the "fast phase" of two successive clearance measurements. For a total blood flow increment of about fourfold, the indicated removal rate of 131-I (K values) changed relatively little.

Both methods were judged to be defective on the following grounds: (a) The mercury-in-rubber strain gauges used for measurement of total blood flow required frequent recalibration when ambient temperature changes were used to influence skin blood flow. (b) The isotope clearance method was not completely free of potential hazard. (c) The exact location of the tracer after its introduction into the skin by ion transfer was uncertain. (d) A measurement period of 30 to 40 minutes was required so that the true slope of the "fast phase", most clearly related to effective capillary blood flow, could be determined. Transition phenomena occurring in the distribution of blood flow during changes of ambient temperature were impossible to study.

Development of temperature-stable forearm blood flow gauges

1. Capacitance gauges. In an attempt to find a new method in which the volume gauge would be relatively little influenced by temperature change, recourse was had to the capacitance method. The mid-portion of the forearm was used as one plate of a concentric capacitor; the other plate was formed by encircling the forearm with a 1/2-inch strip of aluminum foil separated from the skin by a 1/4-inch layer of polyurethane foam, which served as a dielectric. The usual difficulties with capacitance recording were

virtually abolished by the use of the arm-electrode system as a variable capacitor in a stable 250 K-Hertz oscillator circuit applied as the excitation of an ionization discharge in a gas-filled tube. The output of the ionization tube was a phase-sensitive D.C. voltage which was proportional to the change in capacitance produced when the arm segment enlarged.

Details of construction and operation of our first capacitance plethysmograph are given in the Progress Report to the U. S. Army Medical Research and Development Command for July 1962 - June 1963 (25). Design of subsequent improved models, performance analysis and calibration problems are discussed in the Progress Report submitted September 1966 (26) and in Appendix II of this report.

Future development of the capacitance gauge was planned to minimize lead problems and to isolate arm band capacitances from the much larger cable capacitance. This was to be accomplished by incorporating some of the electronic circuitry in the arm band as miniturized, integrated circuits and operational amplifiers. Since over a year of development work and testing would have been involved, these plans were abandoned in favor of a quicker solution.

2. Elastic resistance gauges. In addition to his work on the capacitance gauges, Mr. Timothy O. Clarke had been conducting a parallel study of the properties of the mercury-in-rubber and similar electrolyte paste-in-rubber (Waggoner (27)) gauges (26). He devised for the Waggoner gauge a circuit capable of measuring fractional resistance changes in the gauges that were independent of temperature, and he made the important discovery that if the fractional resistance change can be measured, the fractional blood flow can be obtained from it directly. (See Appendix II).

The relationship between fractional resistance change and fractional blood flow also holds true for mercury-in-rubber gauges. For this relationship to be utilized in practice, two conditions must be observed:

1. The gauge must go around the arm exactly 1.0 times.
2. The device that measures gauge impedance must not be significantly affected by series or shunt impedances in the cables or measuring circuits.

The life of gauges has been greatly extended by using Dow-Corning silastic medical grade tubing instead of natural rubber. Mercury as the resistive element is more durable

than the electrolyte pastes of the Waggoner gauges, the latter tending to dry out with time and to be subject to resistance changes attributable to electrolysis. Details of the improved technique and the circuit for the mercury-in-silastic gauges are given in the Progress Report of 1968 (28) and in Appendix II of this report.

Helium flux through the skin as an index of effective capillary blood flow

A report by Behnke and Willmon in 1941 (29) presented evidence of the transfer of helium through the skin in subjects enclosed in rubber bags from the neck down when the gas phase in the bag was 90 to 95 per cent helium. Helium flux rate was estimated from samples of expired air collected over periods of half an hour. Helium flux rates of the order of 50 ml/1.20 M^2* /hr were characteristic when the temperature in the bag was 22 to 28.5°C. At progressively higher temperatures up to 35.5°C, the rate of helium flux increased linearly with temperature to about 170 ml/1.20 M^2/hr. This observation was in agreement with the early reports of Schierbeck (30) and von Willebrand (31) of a "critical temperature" above which oxygen and carbon dioxide transfer through the skin increased with rising temperature. From values of helium solubility in blood at equilibrium conditions, for a pHe of 700 mm Hg, Behnke and Willmon calculated that a transfer rate of 170 ml of helium per hour would require at least 20 liters of blood circulating through the skin of the neck, trunk and extremities, a figure only slightly higher than the estimates of skin blood flow by Hardy and Soderstrom (32) based upon rates of heat loss from the bodies of nude, motionless men.

The possibility that the rate of helium transfer through the skin could be used as an index of blood flow through the superficial skin capillaries has been explored in our laboratory. Helium offers many advantages over other possible tracer substances. Its solubility in blood and other body fluids is lowest among the commonly available non-toxic gases. It may be administered over long periods of time and repeatedly to the same individual without

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* The surface area of Behnke's subject, minus correction for head and neck and skin surface in contact with the rubber bag.

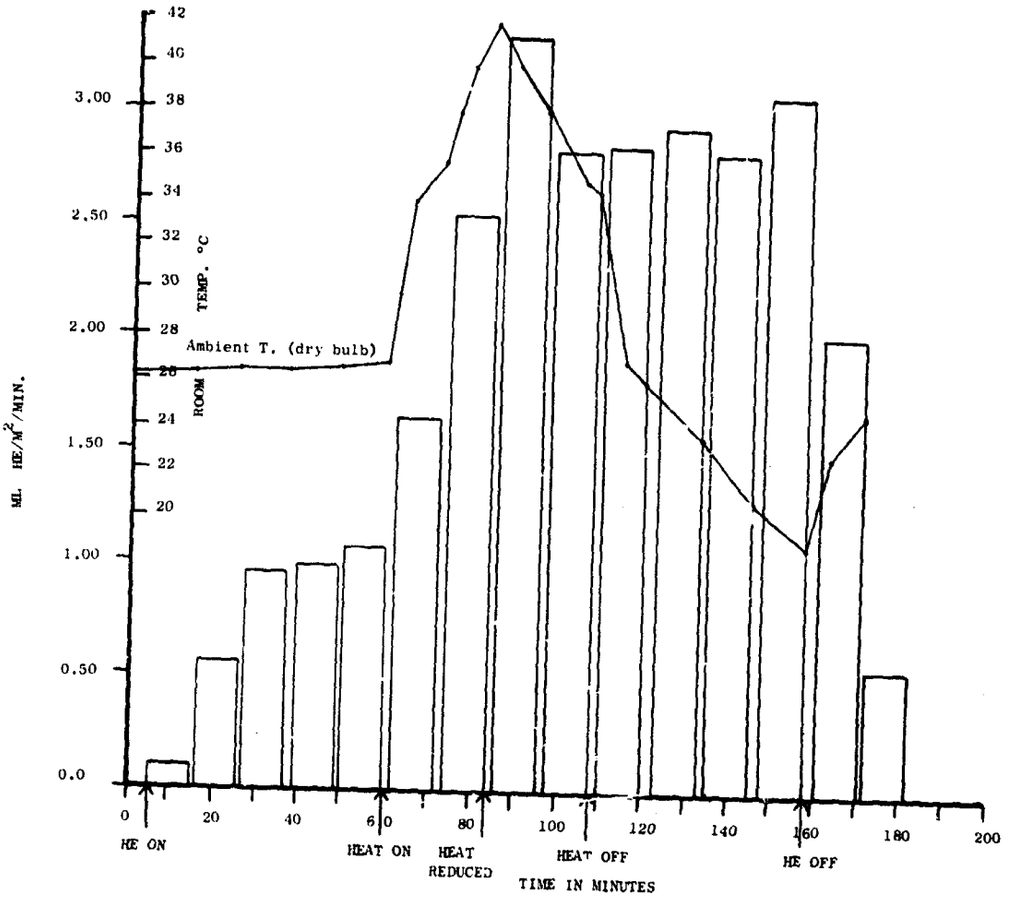

Figure 2-4 Helium leak rates from forearm skin during heating and cooling.

hazard, and thus makes possible the repeated use of trained subjects. Forearm blood flow values obtained from experiments on novice subjects are particularly difficult to evaluate. The pattern of blood flow in the presence of apprehension incident to the novel situation is likely to involve simultaneous vasoconstriction in the skin and vasodilatation in skeletal muscle.

A third advantage favoring helium flux rate as an index of effective capillary blood flow in forearm skin was that the sampling rate could be increased about fourfold over that possible with isotope clearance procedures.

1. Helium analysis by gas chromatography. Rates of helium transfer through the skin were measured by collecting 2-ml air samples from plastic capsules cemented to the forearm skin of subjects who were breathing a mixture of 80 per cent helium and 20 per cent oxygen. The helium content of the collected gas was measured in a gas chromatograph and required that the helium content of samples be not less than 0.25 µl. At low to moderate ambient temperatures, most of our subjects required sampling intervals of 10 minutes in order to accumulate enough helium in the collection capsules for accurate analysis. The response time of the analytical system was of the order of 7 minutes. In practice we adopted a sampling interval of 10 minutes for all of our studies. From the onset of helium breathing, only two samples could be taken before the rate of helium transfer through the skin became essentially constant at constant ambient temperature $\pm$1.5°C. Details of sampling procedures, equipment and helium analysis are given in the Progress Report of September 1966 (26) and in Appendix III of this report.

(a) Effects of heat and cold. The study of a heated subject illustrated in Figure 2-4 shows the time course of changes in helium flux rate (shown as histogram) through the forearm skin of a lightly clothed 22-year-old man reclining at rest for three hours and exposed to indifferent, high and low temperatures. Helium flux rates reached plateau within the first 30 minutes and then rose with the rising ambient temperature. The first helium sample taken after the onset of heating shows the increment of helium flux rate before the onset of visible sweating, which was noted when the ambient temperature reached 35°C.

The persistance of high rates of helium flux through the skin during the period of decreasing environmental temperature was later shown to be relatively independent

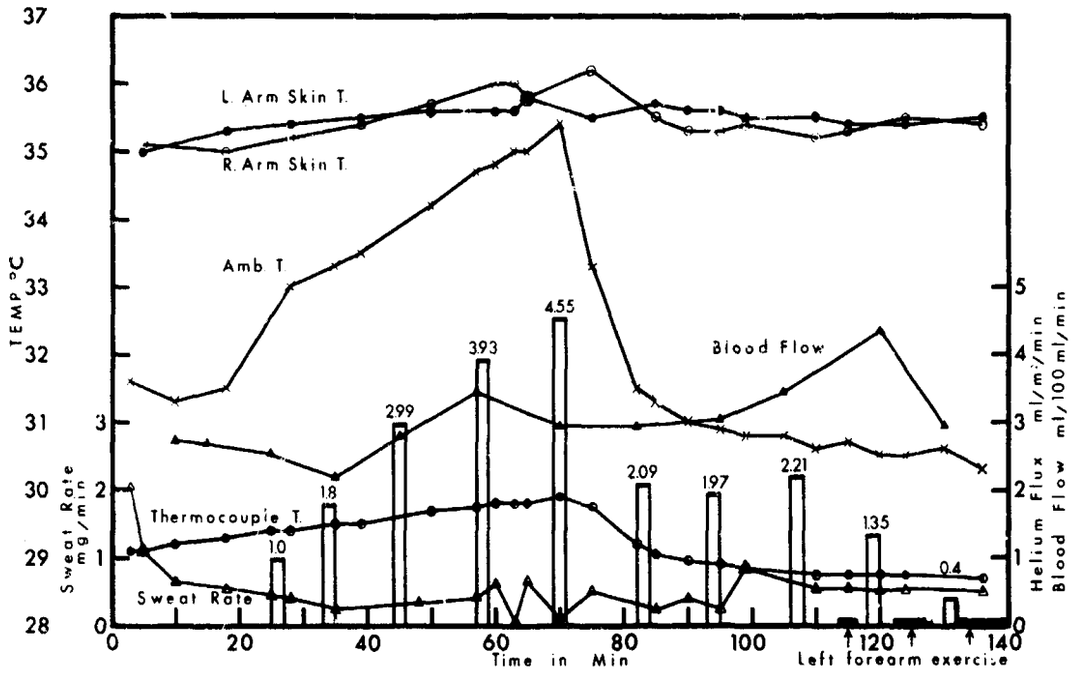

Figure 2-5 Forearm skin temperatures, blood flow, helium flux and sweat rate during moderate general body heating and cooling.

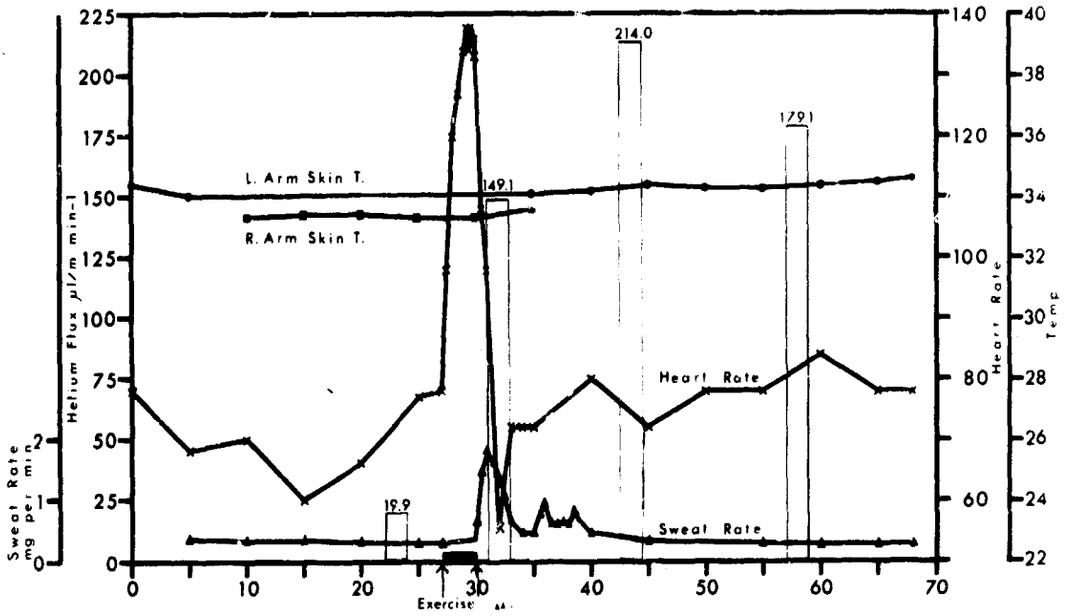

Figure 2-6 Relationship of sweating to helium leak rate after brief, heavy exercise.

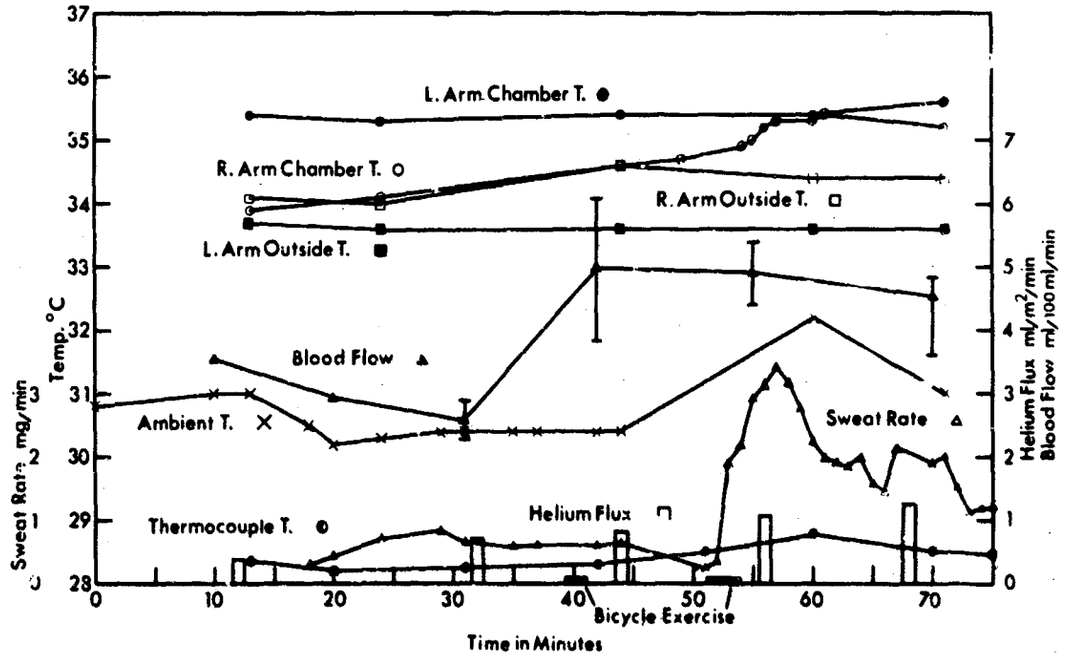

Figure 2-7 Changes in forearm blood flow, skin temperature and rate of transfer of helium through the skin after light exercise and after moderate exercise during increased ambient temperature.

of skin blood flow. Figure 2-5 shows a more detailed study on the same subject, in which the ambient temperature rise was limited to the range in which sweating, measured by the method of Bullard (33) (see also Appendix III), was noted only intermittently. The slight increase of forearm blood flow is consistent with a degree of skin vasodilatation limited to the passive response to decreased adrenergic discharge during the period prior to sweat gland activation.

(b) Effects of posture and exercise. Figure 2-5 also illustrates the effect of redistribution of forearm blood flow by exercise. During the last 25 minutes of the experiment, brief bouts of forearm exercise (fist-clenching, followed by rest periods during which blood flow measurements were made) increased total forearm blood flow, decreased helium flux rate and left sweating and skin temperatures unchanged. The lack of change in skin temperature during the period of declining helium flux rate may be the consequence of constriction of sphincters of the superficial dermal capillaries and hence, a redistribution of flow within the skin without change in total skin blood flow.

Both posture and exercise influence skin blood flow and modify the relationship of helium flux through the skin to sweating. Figure 2-6 presents results of an experiment in which a brief bout of exercise on a bicycle ergometer was used to elicit sweating in a subject equilibrated at an ambient temperature of 26°C. The skin temperatures shown are those measured within the capsules used for collection of helium samples and for continuous measurement of sweat rate. Heart rate was monitored from the recorded output of a cardiotachometer. Sweat rates were recorded from the output of a resistance hygrometer and samples of gas from the plastic chamber attached to the forearm skin were analyzed for helium at intervals of 10 to 15 minutes. The histograms show helium flux rates in $ml/M^2/min$. The subject was seated at rest on the bicycle ergometer. Measurement of sweat rate and breathing of the He/O_2 mixture were begun at 5 minutes, and a pre-exercise sample of gas from the arm chamber was taken. At 27 minutes, the subject began a 3-minute period of exercise at 300 kg M/min. No significant sweating was noted until after the end of the exercise, when the rate rose from 0.4 to 2.0 $mg/10\ cm^2/min$ and returned within 10 minutes to the pre-exercise level. Helium flux rate increased more than 10-fold in the two sampling periods immediately after exercise and was still substantially elevated 30 minutes afterward. The low values of helium

flux rate obtained in this experiment are at the lower range of those commonly found in cool, resting subjects studied in the upright position. The data in this experiment resemble those shown in Figure 2-4 in that elevated rates of helium flux persisted for a considerable period after the episode of sweating.

The same subject was again studied during very light exercise and during moderate exercise. Figure 2-7 illustrates the changes in skin temperature, total forearm blood flow, sweat rate and helium flux in a control period, after 2 minutes of exercise at 200 kg M/min and after 3 minutes at 600 kg M/min. The vertical lines on the last four blood flow measurements indicate the range of flow values obtained in 8 to 10 successive venous collections. The line connecting the solid triangles indicates the position of the arithmetic mean.

The period of light exercise evoked neither sweating nor significant change in the rate of helium transfer through the skin. Sweating increased sharply after 1.5 minutes of heavier exercise, but the magnitude of the increase of helium flux was only about 1 per cent of that seen during similar rates of sweat production by reclining subjects at rest in a hot environment or with sweating induced by sudotropic drugs. Skin temperature within the gas collecting capsule rose approximately 0.5°C after the heavy exercise. It must be assumed that increased blood flow through the skin was responsible for at least part of the larger total forearm blood flow which persisted for at least 16 minutes after the second bout of exercise. For reasons not immediately apparent, the distribution of blood flow in the skin appears to be independent of the total amount of flow under certain circumstances. Heat can be lost through the skin, whether or not the blood circulates primarily through superficial capillaries or deeper channels. The route taken by helium in diffusing from blood through the tissues is not known; it is safe to assume that the largest portion of the helium which finds its way through the surface of the skin has come into equilibrium with helium in interstitial and perhaps cell water, mainly after diffusing through capillary walls.

(c) The effect of arteriovenous anastomoses on cutaneous excretion of helium. In order to examine the effects of large shunt blood flow upon helium flux through the skin, a study was designed to measure simultaneously total blood flow and helium flux rate in a digit and in the forearm of heated subjects. These two sites of measurement were selected to compare the relationship between

blood flow and helium excretion from a skin area rich in arteriovenous anastomoses (finger) with that from a part of the skin considered to have no such vascular shunts (forearm).

Procedure:

In each experiment, the subject rested on a chaise in a temperature-controlled room with both arms and hands supported at heart level by a plywood shelf attached to the chaise arms. Thermistors for measurement of skin temperature were attached to the middle finger of each hand and the mid-portion of each forearm. A plastic capsule for sweat rate measurement by the resistance hygrometry method (33) was attached to one forearm. In a symmetrical position on the opposite forearm, a plastic capsule was attached for collection of helium from the skin surface. Skin temperature within each of these capsules was monitored by spring-loaded thermistors held in contact with the skin (see Appendix III). A mercury-in-rubber strain gauge was attached to one forearm, with the usual wrist and arm cuffs for venous occlusion plethysmography. Finger sweat rate and excretion of helium from finger skin were sampled by enclosing the middle finger of each hand in a plastic capsule sealed to the skin at the crease marking the joint between the proximal and second phalanx. A small pneumatic cuff about the base of the finger carrying the capsule for finger sweat rate measurement permitted intermittent use of the same capsule as an air-conduction plethysmograph. Measurement of finger blood flow was accomplished by switching from the hygrometer air stream to closed communication of the finger chamber with a pressure transducer.

The subject was fitted with a Bennett face mask and at zero time was allowed to breathe a mixture of 80% helium and 20% oxygen. Air samples from the helium collection chambers on finger and forearm were taken every 10 minutes. Blood flow measurements in the finger and forearm were made during a 90-second interval at the mid-point between helium samplings. Procedures for gas sampling and analysis have been given in detail in the Progress Report on this contract of September 1966 (26) and in Appendix III of this report.

Experimental time periods ranged from 58 to 200 minutes in length, during which skin blood flow was increased by increasing temperature of the room from 22.5° to 45°C, dry bulb. No attempt was made to control humidity. A fan provided air movement within the controlled room at about

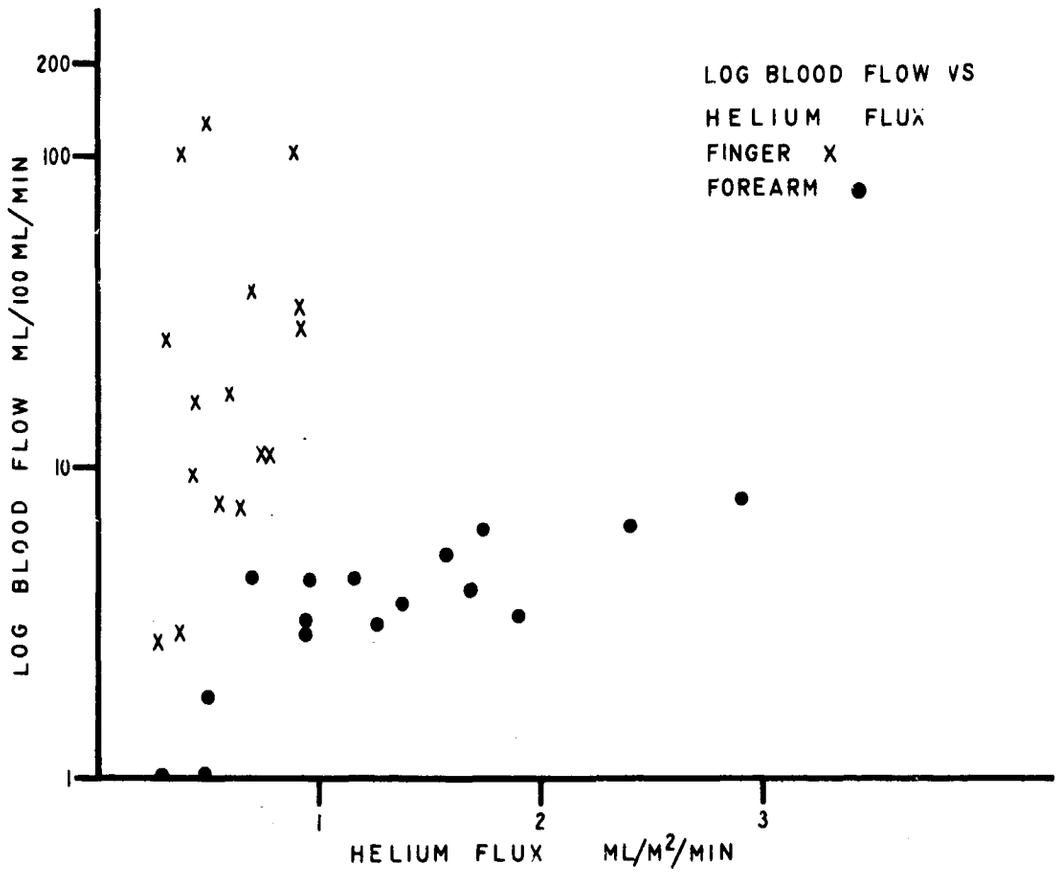

Figure 2-8 The effect of arteriovenous anastomoses on the relation between blood flow and helium flux through the skin.

2 meters per second. All recording and control equipment was outside the room; the subject was isolated except during collection of helium samples and flushing of helium chambers with fresh air.

Results:

Levels of blood flow in the fingers achieved by exposure to heat ranged from 6.0 to 161 ml/100 ml/min. Blood flow levels in the forearm ranged from 1.0 to 8.5 ml/100 ml/min. Both increments of blood flow are considered to represent changes that occurred in skin and subcutaneous tissue (34, 35). The rate of water loss from the finger skin was more variable than that from forearm skin, with only forearm skin yielding water at rates greater than 0.25 $mg/cm^2/min$ at the higher temperatures. Excretion of helium from forearm skin was found to increase with rising skin temperature and blood flow; the most rapid helium excretion occurred during active sweating. Available data do not provide any means of determining to what extent the augmented loss of helium may be attributed to sweat gland activity, as such, or to the increase of skin blood flow which accompanies the onset and maintenance of sweating. Rates of helium excretion from finger skin observed in these experiments ranged from 0.3 to 0.9 $ml/M^2/min$. Excretion rates from forearm skin in the same experiments ranged from 0.3 to 2.9 $ml/M^2/min$.

Data from four experiments are given in Figure 2-8. Owing to the large values reached for finger blood flow, a log scale was used on the axis of ordinates for both finger and forearm blood flow in ml/100 ml/min. Rates of helium excretion from the skin are displayed on an arithmetic scale on the axis of abscissae. Each cross on the graph marks the excretion rate of helium from finger skin at the rate of finger blood flow measured during that helium collection period. The solid dots mark rates of helium excretion from forearm skin and the corresponding forearm blood flow rates.

Discussion:

The data show that rates of helium excretion from finger skin remained relatively fixed while finger blood flow increased more than 25-fold during exposure to a moderately hot environment. This pattern of response is consistent with the hypothesis that nearly all of the blood flow increment was carried by arteriovenous anastomoses and thus was out of free diffusion communication with the skin surface. An alternative interpretation might be that

some kind of "diffusion barrier" in finger skin was responsible for the dissociation between blood flow and the rate of helium loss through the skin. Against such a possibility is the observation that the time of onset of helium excretion from the skin of the finger and of the forearm was nearly identical, as was the time course of decrease of helium excretion following the restoration of room air as the breathing mixture.

The nearly linear relationship between the rate of excretion of helium and the forearm blood flow suggests that superficial dermal capillaries share in the blood flow increase that accompanies exposure to heat. A recent summary of the relationship between blood flow and transcapillary exchange indicates that both increased blood flow in capillaries and increased total capillary area are required for increased exchange by diffusion (36). It seems unlikely that significant increases in the amount of flowing blood carried by a capillary bed could be achieved by dilatation of a fixed number of these vessels. An increase in the number of open capillaries seems much the more likely process by which an increased blood content and blood flow through superficial capillaries occurs. Active vasomotion has been described in capillaries under the capillary microscope in the superficial layers of the skin (37). Alternation of flow in adjacent capillaries and wide variations in the number of open capillaries appear to be time-independent of tonus changes in the supplying arterioles (38, 39). In most tissues, control of precapillary sphincters by chemical (metabolic) autoregulation takes precedence over autonomic nervous control and over myogenic autoregulation. Whether the same is true in skin, a tissue in which only about 10% of its bulk consists of cells, cannot be decided on the basis of the evidence now available.

(d) Analysis of helium in blood; in vitro studies. The blood nitrogen method of Farhi and his associates (40) was modified and adapted for analysis of helium concentrations in blood. The original method required only the vacuum extraction of gases in a Van Slyke apparatus from 1.5-ml blood samples and the delivery of the extracted gases into a gas chromatograph equipped with a trapping column for removal of water vapor and carbon dioxide followed by a 4' column of 5A molecular sieve. Helium was used as the carrier and reference gas. Our gas chromatograph, which uses air as the carrier gas, produced linear peak heights when used for analyses of helium in water, but we were unable to obtain accurate results with blood samples. The

large amount of oxygen in the gas extracted from blood was the source of the error. Incomplete separation of the oxygen and helium on the 10' column caused falsely high "helium" peaks in the chromatograph record. We have modified the original method by using chemical absorption of oxygen with the standard reagent used in Van Slyke analyses. Improved delivery of gas from the Van Slyke apparatus was achieved by substituting a linear switching valve for the four-way glass stopcock described by Farhi, et al.

Analyses of samples of distilled water equilibrated in a tonometer for 30 minutes at 37°C with pure helium and with mixtures of various concentrations of helium in air yielded values of helium within 0.5 - 1.0% of the solubility values given in standard tables (41). Although others have stated that the solubility of helium in blood is the same as that in water (42, 43), Edwards et al. (42) reported that they obtained different solubilities with each blood they measured and found it necessary to make tonometric studies on each individual's blood as a basis for comparison with arterial blood samples. Our analyses of outdated bank blood equilibrated with various concentrations of helium in air at 37°C yielded values about 9 to 10% below values calculated on the basis of a Bunsen coefficient of 0.0075 ml/ml STPD Since the solvent phase for helium in whole blood is chiefly water, we recalculated our data on the basis of blood water determined gravimetrically on 1.0 ml aliquots of each blood equilibrated and found helium solubilities per milliliter of blood water to be within ± 2% of the expected values.

For such an inert gas as nitrogen or argon, the steady state of inhaled gas distribution may be defined as that state in which the gas is uniformly distributed in the lungs and in which no exchange of that gas is occurring between alveolar gas and blood. For a given perfusion rate the saturation speed of a gas in a particular tissue is inversely proportional to the square root of the molecular weight of the gas and is independent of the solubility (43). In a true steady state, in the case of nitrogen, the conditions imply no exchange between blood and tissues and no exchange between the blood circulating in the superficial capillaries and the surrounding atmosphere. In an individual breathing 80% helium and 20% oxygen, the rising partial pressure of helium in the skin establishes a substantial diffusion gradient against the negligible partial pressure of helium in ambient air. Helium excretion from the skin at approximately 1 to 2 $ml/M^2 \cdot min^{-1}$ is the usual finding at skin temperatures between 27° and 33°C. It is unlikely that concentrations of helium in mixed venous blood would ever reach values exactly equal to those found in arterial blood as long as the individual continued to breathe the He/O_2 mixture.

(e) Analyses of helium in blood; in vivo studies. Helium concentrations in blood samples were taken over a 30-minute period during which a 59-year-old subject breathed a mixture of 80% helium and 20% oxygen. Preparation for the experiment consisted of placing indwelling catheters through G 19 needles. One catheter was inserted into a deep branch of the median cephalic vein at the flexure of the elbow. Another was inserted distally into a superficial branch of the brachiocephalic vein in the midportion of the forearm and a third distally into the largest central branch of the dorsal venous arch on the back of the hand. A pneumatic cuff was placed around the wrist and connected through a solenoid valve to a 6-liter tank pressurized at 240 mm Hg. The purpose of the wrist cuff was to permit sampling of blood from the superficial cutaneous vein of the forearm without danger of having the blood sample contaminated by blood from the hand. A 5-ml sample of blood from the deep vein catheter was drawn for analysis in triplicate of blood water content while the subject was seated at rest in a temperature-controlled room at 31°C dry bulb. All catheters were flushed with heparinized, sterile saline and plugged. A Bennett face mask was used to administer the breathing mixture of He/O_2. Over a 30-minute period, 5-ml blood samples were taken from the deep vein at 5, 10, 20 and 30 minutes. At 30 minutes a 5-ml sample of blood was also drawn from the dorsal hand vein after first thoroughly warming the hand in water at 42°C. Three minutes after the hand vein sample, the wrist cuff was inflated to isolate the hand, and a 5-ml blood sample was drawn from the superficial forearm vein. Analysis of the samples for helium was begun as soon as the first sample was available. Each helium analysis required about 12 minutes. Syringes containing the subsequent samples were sealed with mercury and stored in an ice bath until they could be analysed. The data are given in Table 2-1.

Table 2-1

Concentrations of helium in peripheral venous blood and "arterialized" hand venous blood during 33 minutes of breathing 80% helium and 20% oxygen.

Minutes after starting $He-O_2$	Source of sample	Helium concentration µl He/ml blood water
5	Deep arm vein	0.78
10	Deep arm vein	0.89
20	Deep arm vein	1.88
30	Deep arm vein	2.26
30	Hand vein ("arterialized")	3.26
33	Superficial forearm vein	0.20

If we use the Fick principle to estimate the minimum blood flow contributing helium for excretion through the skin by means of the following equation:

$$\dot{Q} = \frac{jHe}{A_{He} - V_{He}}$$

where $\dot{Q}$ = Minimum blood flow in $ml/M^2\ min^{-1}$ (minimum flow required to carry the helium excreted through 1 M^2 in 1 min).

jHe = rate of helium excretion through skin in $ml/M^2\ min^{-1}$

A_{He} = helium concentration in "arterialized" venous blood in µl of helium/ml blood water

V_{He} = helium concentration in superficial cutaneous venous blood in µl of helium/ml blood water

for helium excretion rates from 0.87 ml to 1.6 $ml/M^2\ min^{-1}$ the respective values for minimum "effective" blood flow in skin are 284 to 505 $ml/M^2\ min^{-1}$.

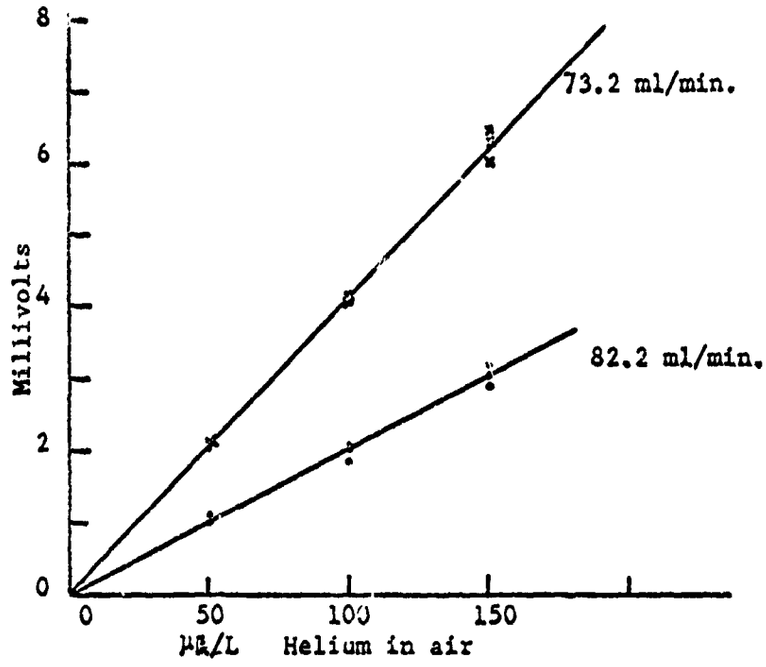

Figure 3-1 Helium concentrations of test gas vs millivolts output at 2 rates of air flow.

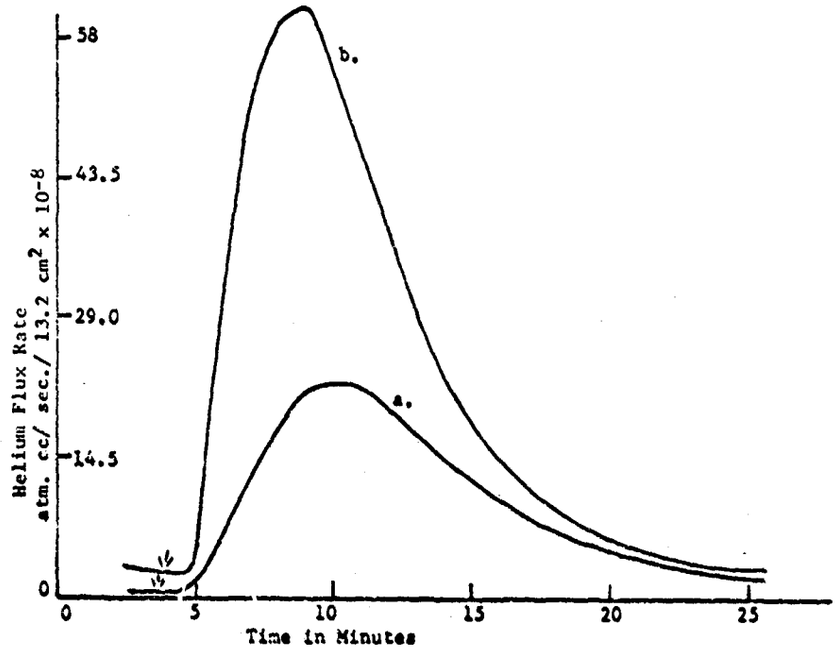

Figure 3-2 (a) Helium leak rate through forearm skin after breathing 1 liter of 80% He and 20% O_2. Skin T: 31.4°C. (b) Helium leak rate following heating of forearm. Skin T: 35.7°C.

PART 3

Continuous Measurement of Helium Leak Rate

1. Helium analysis by means of a Helium Leak Tester. With helium analysis by gas chromatograph our studies of the distribution of blood flow within the skin were limited, for all practical purposes, to steady states. Collection periods of 10 minutes and an additional 10 to 12 minutes for analysis prolonged each experimental session. Subjects were required to wear a face mask for periods as long as six hours. The discomfort of wearing the mask and the long intervals without moving were obviously serving as stimuli capable of altering the distribution of blood flow.

A Nier type focusing mass spectrometer was used by Adamczyk and his associates (44) for quantitative measurements of carbon dioxide, neon, helium, nitrogen and argon through skin. Air was passed continuously through a collection capsule attached to the skin. The area sampled was about 12 cm^2, and the air flow rate was about 60 $\mu l/sec$. Analytical ranges covered helium flux rates from 0 to 75 x 10^{-4} $\mu l/cm^2/sec$, an increase of several orders of magnitude in sensitivity over that of our previous procedure. An additional important advantage was that sampling was continuous rather than at relatively long intervals.

It occurred to us that the advantages of the mass spectrometer principle might be used in our studies for about 1/5 the cost of the least expensive mass spectrometers if a helium leak tester could be adapted for this purpose. A Model LD-100 Varian Helium Leak Tester yielded promising results in preliminary testing.

Special procedures and equipment devised for continuous sampling and analysis of helium collected from the skin of subjects breathing He/O_2 mixture are given in Appendix III, 2 of this report and in the Progress Report submitted to the U. S. Army Medical Research and Development command October 1968 (28).

2. Performance characteristics

(a) Linearity: The relationship between helium concentration and the indicated voltage output from the leak tester shown in figure 3-1 supports the concept that helium partial pressure in the fraction of the sample presented to the analyzing tube is linearly dependent upon the partial pressure of helium in the original sample. The conditions

for molecular flow are usually considered to apply only in the stationary state (45). It is evident that the flow rate of the sample must remain constant for the analyzed fraction of the sample to fit the case for molecular flow. When the sampling flow rate was increased from 73.2 ml/min to 82.2 ml/min, the slope of the linear regression was approximately halved.

(b) Time characteristics: The time elapsing between administration of helium to a subject and the initial rise of the recorder trace showing some helium leaking from the skin varies with the age of the subject, skin temperature and blood flow, and sweat gland activity. In young men 19 to 30 years old, the interval is as short as 30 seconds. In one subject 61 years old the shortest time measured during active sweating was 47 seconds and the longest, measured at 20°C, R.H. 35%, was 160 seconds. This latency of response is the sum of a large number of factors, each of which is subject to change by the conditions of the experiment. Figure 3-2 was made by tracing two response curves from a potentiometer chart recorded during an experiment on a resting young subject. The responses were obtained from successive recordings 30 minutes apart but are shown on the same time scale in the illustration for convenience of comparison. Arrows mark the time of administration of 1 liter of helium-oxygen mixture. The ambient temperature was 25°C and the relative humidity 37% during both measurements. An electric heating pad was wrapped around the forearm upon which the helium collecting chamber had been secured. Curve (a) was recorded at a skin temperature of 31.4°C with no current flowing to the heating pad. Curve (b) was recorded after a new inhalation of one liter of helium-oxygen mixture and 15 minutes after the beginning of moderate heating. The skin temperature under the heating pad was 35.7°C at the peak of helium flux. The ordinate scale on figure 3-2 has marks at intervals of 10 chart divisions each. The numbers of each mark are direct conversions of chart divisions into helium flux rates, having the dimensions of atmosphere cc/sec x 10^{-8}, corrected to a sampling area of 13.2 cm^2. The difference in time between the administration of helium and the initial part of the trace rise is small compared to the considerable difference in times required to reach peak height. Precise measurement of onset times is not possible, but the longer of the two is not more than a minute and the difference in onset times is of the order of 10 seconds or less. The time to peak height is 6.5 minutes in curve (a) and 5.2 minutes in curve (b). Since there is no reason to suppose that there was a significant difference in arterial helium saturation or cardiac output in the two tests, it is reasonable to assume that superficial skin blood flow and capillary

area available for exchange participated in generating the steeper rise to peak level seen in curve (b) (46).

Response times of the helium leak tester and of the recorder are respectively 10 sec and 0.5 sec. These intervals are small and, as far as we can determine, not altered by any of the conditions of experiment we have explored. When the system under study includes the sampling system, the latency interval is increased by only 8.3 msec by the addition of the transit time for gas from the collecting chamber on the skin to the outer surface of the "molecular leak" when the measured flow rate is 78 ml/min.

3. Effects of circulatory reflexes associated with changes in posture upon the distribution of blood flow in the skin. The reduction of forearm blood flow induced by active or passive change from the reclining to the upright position has been documented and summarized by Abramson (47). The mechanism of the reduction of blood flow has been said to involve constriction of resistance vessels in skeletal muscle (48). The evidence for this view was the finding of a progressive decline of oxygen saturation in blood taken from deep forearm veins. Skin blood flow variations during postural change have been less clearly defined. Declines of skin temperature during upright tilt have been presumed to indicate decreased blood flow in the skin, but control of ambient temperature was not mentioned nor whether a novice or veteran subject was being used in any given experiment (49). Nielsen (50) and Scott (51) have reported on the effects of ambient temperature, acclimatization to heat and experience of the subject with the procedure upon the magnitude of the skin temperature response to tilt.

The experiments reported here were designed to determine the conditions under which requirements of temperature regulation may take precedence over general circulatory reflexes concerned with maintenance of total peripheral resistance during episodes of diminished cardiac output. We have chosen, as the simplest and safest means of manipulating cardiac output, the simulation of upright posture by means of lower body negative pressure (LBNP). This method was preferred to the tilt table because it affords freedom from disturbance of equipment for measuring blood flow, helium leak rate, skin temperature and sweat rate. By measuring the rate of leakage of helium through the skin and total forearm blood flow, we hoped to be able to assess the time course of changes of blood flow to the skin as well as the distribution of blood flow within the skin.

Procedure:

The purpose of this group of experiments was to examine the changes in total forearm blood flow and the changes of blood flow in the superficial layers of the skin during a 5-minute period of simulated upright posture produced by LBNP. The equipment used for the application of LBNP was similar to that described by Brown et al. (52). The subject, wearing socks, track shorts and a loosely fitting surgical scrub shirt, reclined on a narrow table with his lower body from the iliac crests to the soles of the feet enclosed in a tunnel made of 20-gauge perforated steel sheet, supported by a solid 1/2" plywood arch at the foot and tunnel arches at the mid-point and upper end. The entire structure was enclosed in a thick (.012") vinyl sheet, which was folded at the sides and bottom for closure and applied smoothly about the subject's trunk up to the level of the 12th ribs. Two heavy polyvinyl tubes 1-1/4" I.D. were cemented with flanges through holes in the vinyl sheet and afforded connection to a household vacuum cleaner and to an adjustable relief valve. A P 23 D Statham pressure strain gauge and Hg manometer were used for continuous recording and visual monitoring of chamber pressure.

A mercury-in-silastic strain gauge was applied to one forearm for measurement of total forearm blood flow with a wrist pressure cuff to exclude hand blood flow and a low-pressure cuff on the brachium for application of venous occlusion. Details of the signal conditioning and control circuits for the arm volume gauges have been described in Appendix II. On the opposite arm were mounted a plastic chamber for sweat sampling by resistance hygrometry and a flat metal chamber for collecting helium from the surface of the skin (see Appendix III, fig. III-12A). Skin temperature was measured through thermistors, one inside the sweat collection chamber and another secured to the skin with adhesive tape close to the sweat collecting chamber.

A Bennett face mask was secured to the subject and connected to a demand valve supplied by house air from a remote source. During periods of measurement of helium leak rate through the skin, the breathing supply was switched manually to a second demand regulator supplied from a tank of 80% helium and 20% oxygen. The helium sampling chamber covered an area of 13.1 cm^2 of skin that was swept continuously by dried house air at 0.5 ml/sec. The air stream was delivered to a mass spectrometer-type

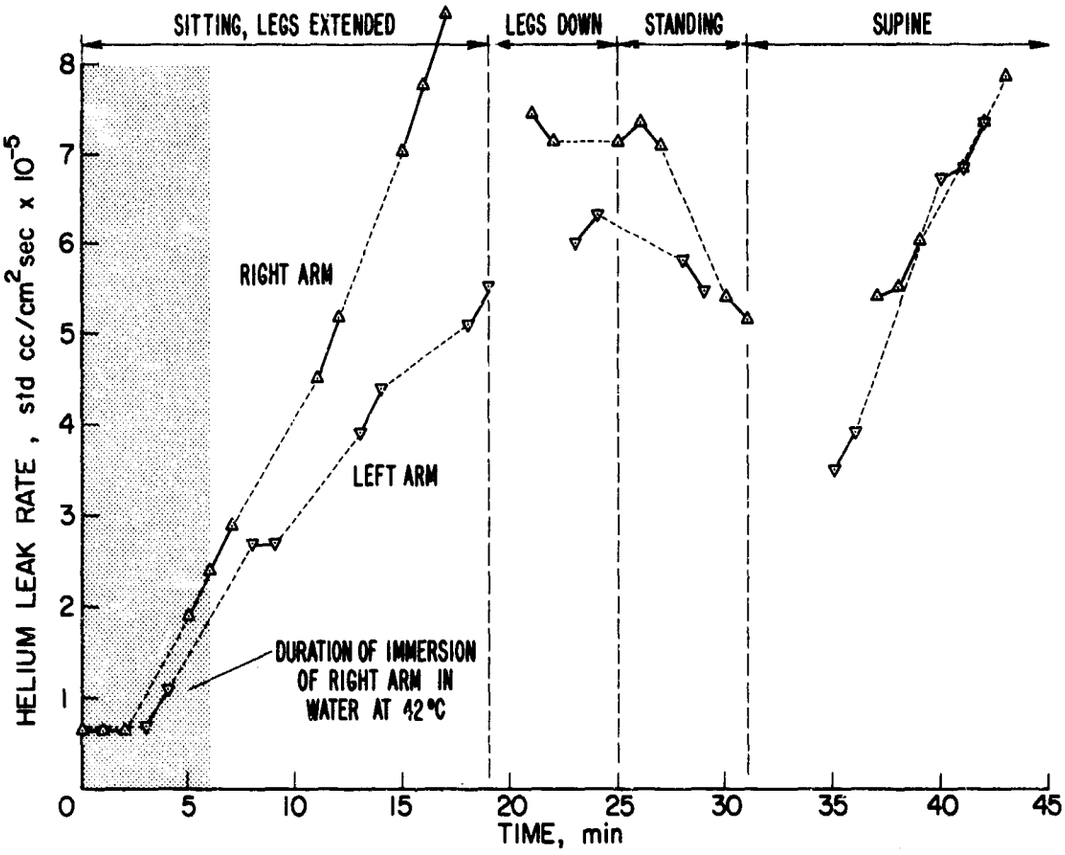

Figure 3-3 Helium leak rates after heating right forearm and after postural changes.

helium leak tester. Details of the sampling and analytical method are given in Appendix III.

After a 30-minute equilibration period at ambient pressure and 25 to 26°C, preliminary records were made of forearm blood flow, sweat rate and skin temperature. In most of the experiments, the breathing mixture was air during equilibration and initial measurements followed by 15 minutes of breathing the He/O_2 mixture. At 5 to 7 minutes after the start of He/O_2 breathing, venous collections were begun to measure forearm blood flow, and the LBNP was initiated and rapidly adjusted to the desired subatmospheric pressure by means of a variable transformer in the power supply to the vacuum source. The LBNP was maintained for 5 minutes in some experiments and for 10 to 15 minutes in others. Recording of helium leak rate was continuous. Blood flow was recorded from about one minute before the onset of subatmospheric pressure until shortly after ambient pressure was restored.

Temperature data were sampled once each minute from the room temperature thermistor and from each of the skin thermistors by an electronic timing circuit. Temperatures, output of the arm volume gauge, resistance hygrometer sensors, and strain gauge pressure transducer used for recording the lower body chamber pressures were recorded on separate channels of a Model 5A Grass polygraph. The output of the helium leak tester was recorded separately on a potentiometric Texas Instrument Servo-riter or Varian Model G 10 recorder.

Results and Discussion:

Prior to the adoption of the LBNP method of inducing "postural" effects upon the peripheral circulation, a test was made to determine the effects of active changes in posture, limiting the measurement to helium leak rate from the skin. Figure 3-3 illustrates alternate measurements from each forearm of a subject during 43 minutes of breathing the He/O_2 mixture. The experiment started with the subject seated with legs extended on a chaise at 25°C. The right arm and hand were immersed in water at 42°C for 6 minutes to test the responses of the recording system. The subject then turned to the side of the chaise and sat with his legs down for 6 minutes. He then rose and stood quietly with his arms at heart level for 6 minutes. The remainder of the time was spent in the supine position. Owing to the presence of helium sampling chambers on both forearms with relatively stiff steel tubing connecting the

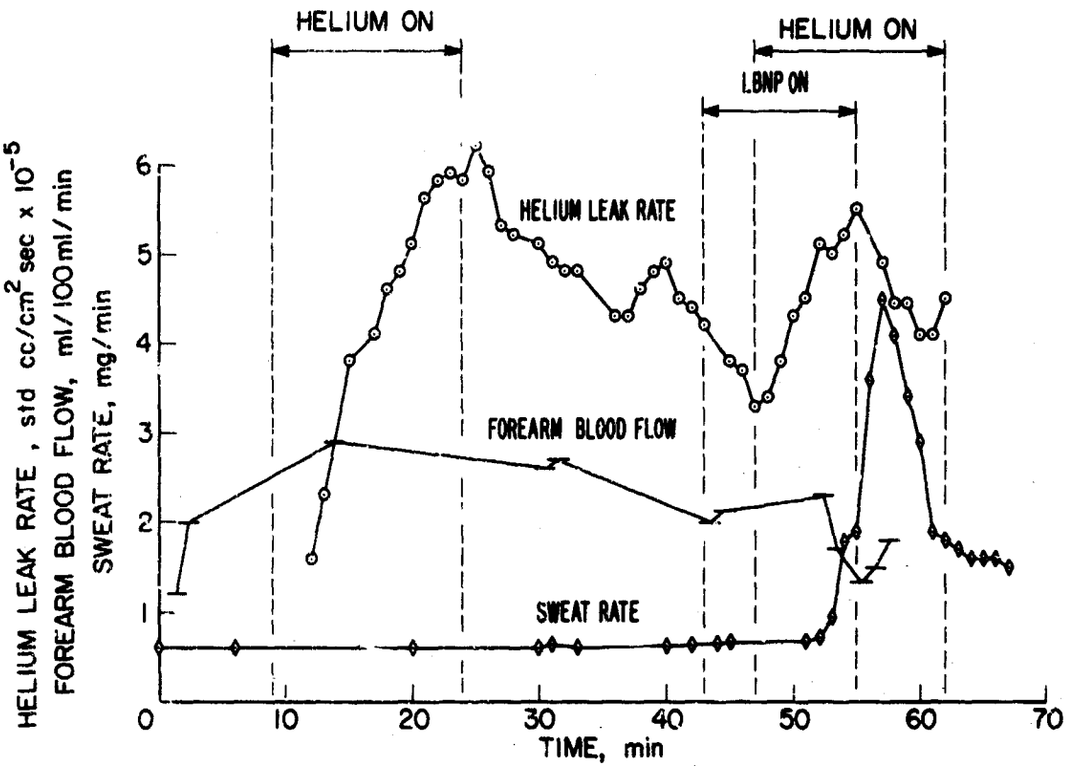

Figure 3-4 Helium leak rates and forearm blood flow rates during a pre-syncopal episode induced by LBNP.

sampling chamber to the helium sensor, each change of position involved an inordinate amount of muscular activity. The recorded signals were too noisy to yield reliable readings for 2 to 4 minutes. The periods of noisy signals are indicated on the graph by long breaks; the dashed portions of each line connect points that bridge the intervals when the sampling valve was set to deliver the air flow from the opposite arm to the helium sensor.

The effect of local heating upon helium leakage through the skin is shown to persist until the first change of posture. If arterial saturation with helium is assumed to be at least 85 per cent complete at 18 minutes after the onset of helium breathing, the amount of extra blood required to furnish the additional amount of helium leaking from the right arm over that from the left would be 0.32 ml/cm^2 min.

The amount of muscular activity and the moderately increased effect of gravity on the circulation produced by sitting with the legs dependent appeared to be sufficient to diminish the rate of increase in leakage -- more in the right (heated) arm than in the left. Quiet standing caused still greater reduction in helium loss through the skin. Reclining supine produced an increased rate of helium leakage that became essentially equal in the two forearms.

Two to five minutes of successful recording were lost during the activity involved in each postural change and thus precluded the use of active postural change for detailed examination of the transitions from one position to another.

(a) Pre-syncopal sweating and reversal of usual blood flow-helium leakage relationships

Recordings were made of forearm blood flow, helium leak rate and sweat rate on subject TD during the first indoctrination session in preparation for subsequent procedures with LBNP. Figure 3-4 shows a preliminary 15-minute period of breathing the helium mixture without application of LBNP, a 19-minute rest period and the effects of 12 minutes of LBNP at 50 mm Hg below ambient pressure.

The first indication of the subject's pre-syncopal state was the development of facial pallor 7.5 minutes after the onset of LBNP. This was shortly followed by a small increase in sweating on the face and hands and then by an indicated increase of sweat output from the forearm. The

ensuing decrease of forearm blood flow was accompanied by only a brief decrease of helium leak rate followed by an increase that persisted during a simultaneous decrease of forearm blood flow. The restoration of ambient pressure was promptly followed by increased forearm blood flow and a simultaneous decrease in the rate of helium leakage. Skin temperature on the left forearm rose from 34.0°C to 34.2°C in the first 2 minutes of LBNP and returned to 34.0°C where it remained for the next 12 minutes. The absence of significant skin temperature change during the fluctuations in total forearm blood flow suggest that most of the variation in blood flow occurred in skeletal muscle, a response shown by Roddie et al. (48) to coincide with a decrease in oxygen saturation of blood taken from deep forearm veins of subjects tilted to the head-up position.

Ambient temperature was 28°C (minus 0.1 to plus 0.6°C) throughout the experiment. Temperature of the air surrounding the lower body was not measured nor was skin temperature of thighs or legs recorded. The subject spent 43 minutes with the lower part of his body enclosed in an unventilated space except for the minor amount of air flow that may have occurred during the application of LBNP. It is impossible to exclude decreased total heat loss as a factor contributing to the burst of sweating toward the end of the period of LBNP. Although a marked increase of skin blood flow usually accompanies sweating, it is not possible to detect it plethysmographically in the presence of simultaneous reductions of muscle blood flow in the same segment of forearm. Helium leak rate was brisk immediately before the onset of sweating and only briefly interrupted in the early part of the decrease in forearm blood flow.

(b) Effects of brief periods of LBNP at -40 mm Hg in the non-sweating subject

In order to determine the changes produced in the superficial skin blood flow and its distribution by simulated upright position in the absence of sweating, the procedure was changed. All subjects were thoroughly familiarized with the procedures. Room temperature was maintained at 25.5 ± 0.5°C. The LBNP chamber was ventilated between applications of LBNP by running the vacuum source at low speed with the vent open to the room. In order to avoid the complications of pre-syncopal changes in the circulation, the low pressure was kept at -40 mm Hg, and the duration was kept to 5 minutes or less. The breathing mixture was switched from air to He/O_2 and recording of helium leak rate from the skin of one forearm

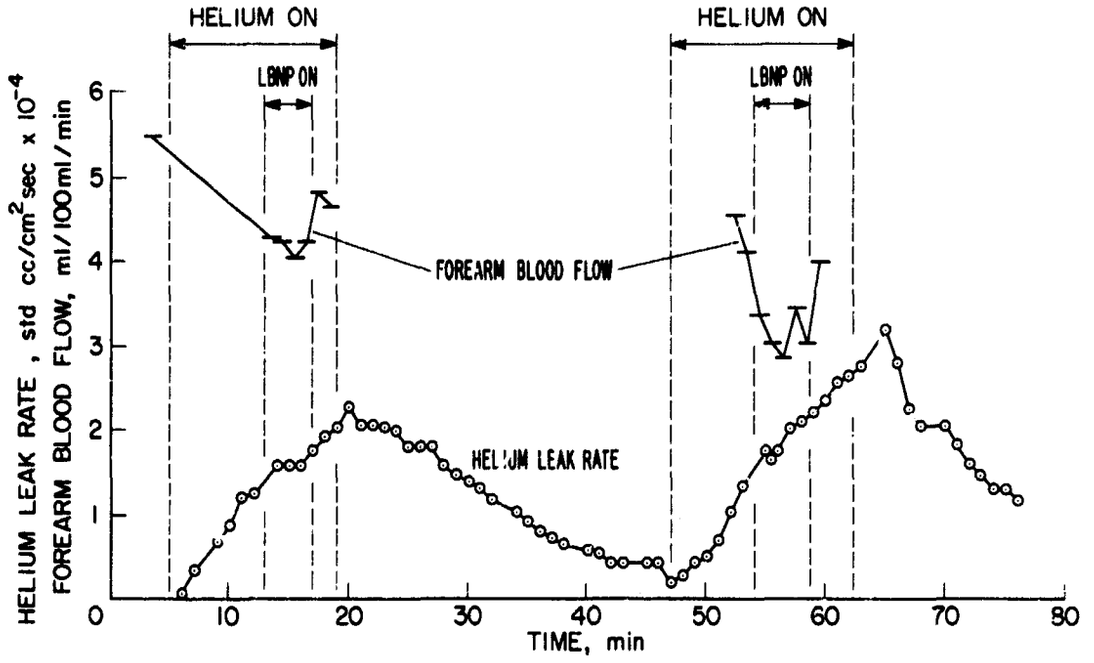

Figure 3-5 Effect of brief periods of LBNP on helium leak rate and forearm blood flow. Application of LBNP 8 minutes after start of helium breathing.

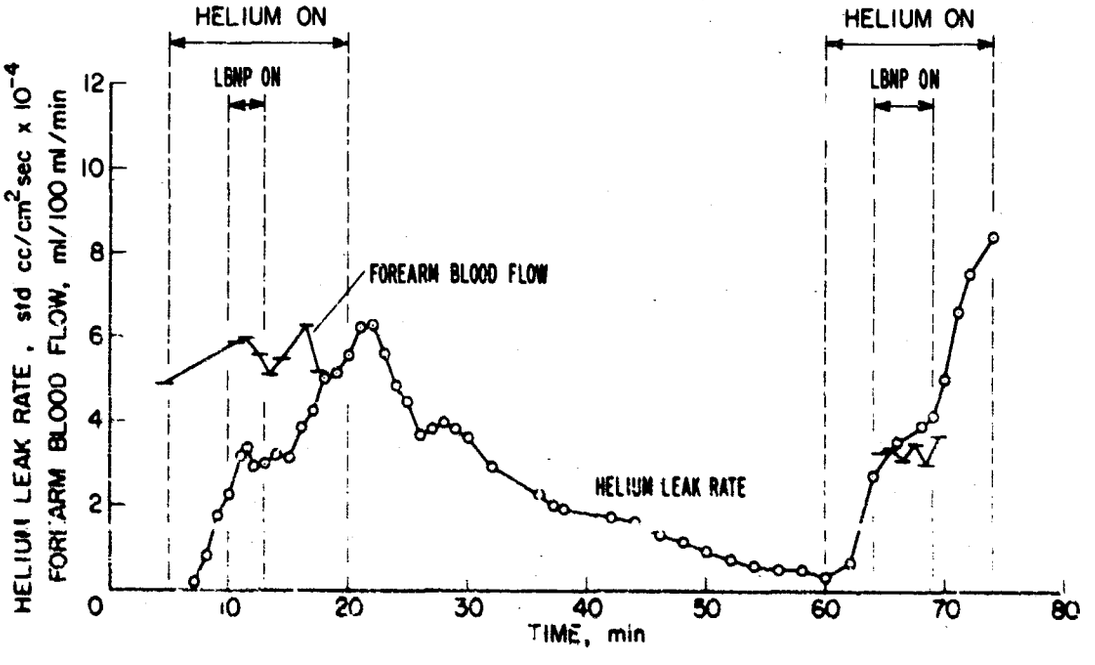

Figure 3-6 Same measurements as in figure 3-5 but with earlier application of LBNP.

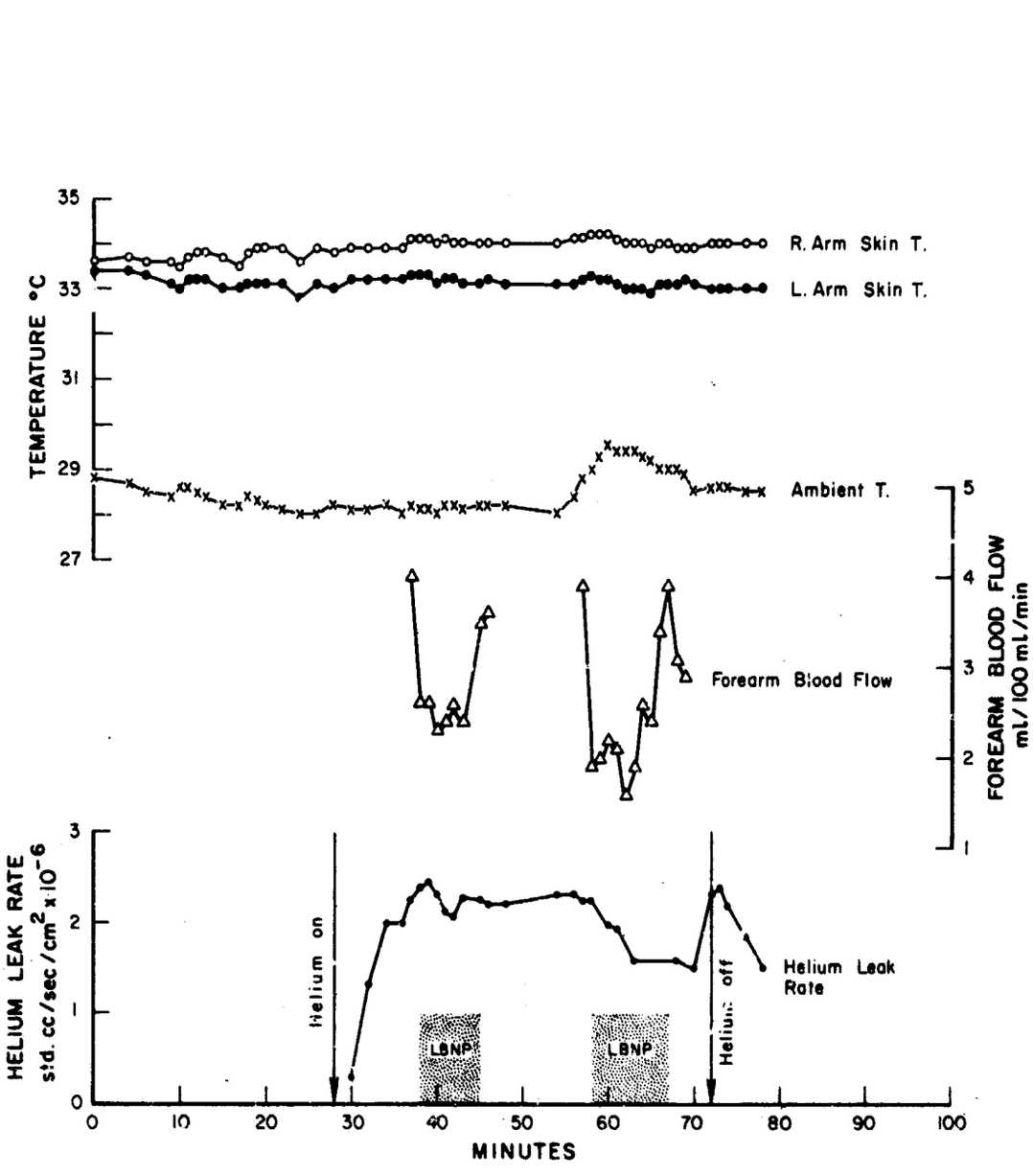

Figure 3-7 Changes in skin temperature, forearm blood flow and helium leak rate during longer periods of LBNP in plateau phase of helium leakage.

was begun. When the rate of helium leakage was well developed, measurements of forearm blood flow were started and continued for 6 to 8 minutes. The vent was closed, and the speed of the vacuum pump was increased rapidly until the pressure within the lower body chamber reached -40 mm Hg. Low pressure was maintained for periods of 3 to 5 minutes, and then ambient pressure was quickly restored.

In four experiments illustrated in figures 3-5 and 3-6, clear cut decreases of blood flow were obtained in three. In all four experiments, the rising rate of helium leakage was interrupted by a period of slower increase, a flat plateau or an actual decrease in helium leakage. All of these changes appeared 60 to 90 seconds after the onset of LBNP; the resumption of rapid loss of helium through the skin began 60 seconds after the restoration of ambient pressure in the experiments shown in figure 3-5 and 1 to 3 minutes before the end of LBNP in those shown in figure 3-6.

In the experiments shown in figures 3-5 and 3-6, the LBNP was applied 10 to 15 minutes after the beginning of helium breathing, i.e. during the phase of rapid increase of helium leak rate. Four additional experiments were carried out to determine the response to LBNP applied during the plateau phase of helium leakage from the skin. Slightly higher ambient temperatures were maintained: 28°C for the first application of LBNP and 29.5° for the second. Figure 3-7 shows data from a representative experiment from this group. In spite of the subject's previous experience with the procedures (at least 10 sessions with measurements of blood flow and helium leak rate and six involving LBNP), his "alerting reaction" pattern is demonstrated by the slight increase of skin temperature in both forearms, the sharp fall in forearm blood flow and the increase of helium leak rate that all occurred just before the onset of LBNP. A similar anticipatory response cannot be distinguished before the second application of LBNP because the room temperature was being increased from the 54th to the 60th minute and could have accounted for all changes before LBNP except the sharp fall in forearm blood flow.

A fleeting decline of skin temperature can be seen in the first LBNP period but is not distinguishable from similar variations caused by random vasomotor changes in the control period. The slightly larger and more prolonged fall in skin temperature seen in the second LBNP period is probably real, though small in magnitude. Helium leak rate changes were larger and longer in duration than those

elicited during the rapid-increase phase of helium leak rate.

The mechanism by which these changes in helium leak rate were produced cannot be positively identified from these data. The small decreases in skin temperature suggest that minute volume blood flow through the skin was changed very little. If the transfer of helium through the skin is a diffusion-limited process as Klocke and his associates have suggested (53), a redistribution of skin blood flow by closure of some precapillary sphincters could reduce the capillary area available for diffusion without much reduction of skin blood flow. Strong vasoconstrictor response in skin resistance vessels does not appear to have been a part of the pattern of increased total peripheral resistance.

4. The relationship of minute volume and distribution of skin blood flow to processes of heat loss. Blood flow increases in the extremities of resting subjects during general body heating. Available evidence indicates that all of the increment is in skin blood flow (10). Two stages of increased flow are identifiable. With heating just short of that capable of evoking sweat secretion, forearm blood flow in most subjects rises to about twice the flow measured when the environmental temperature was cool (about 18°C at relative humidity less than 55 per cent). As ambient temperature and humidity are raised, skin blood flow increases more rapidly. The augmented skin blood flow was shown to coincide with the onset of sweating (54). Sympathectomy or blockade of cutaneous nerves, through which sympathetic nerve fibers pass to the blood vessels and sweat glands, increases by about twofold the forearm blood flow of subjects resting at 25°C. General body heating causes no further increase of blood flow in the sympathectomized or nerve-blocked forearm, and sweating does not occur in the blocked region (34).

The first stage of vasodilatation appears to be the result of central inhibition of adrenergic sympathetic nerve discharge: a passive consequence of relaxation of pre-existing vasomotor tone in some of the resistance vessels of the skin. The second stage of vasodilatation and sweating are both absent after sympathectomy or nerve block with local anesthetic. Since nervous excitation is required for the degree of vasodilatation yielding maximum blood flow rates in response to general body heating, the mechanism appears to involve either an "active" (neurogenic) vasodilatation or a secondary consequence of activation of

sweat glands by sympathetic cholinergic fibers.

Fox et al. (6) have presented evidence suggesting that bradykinin, formed by an enzyme released by active eccrine sweat glands, may be the vasodilator transmitter substance. Evidence supporting this view rests upon the finding of a polypeptide having pharmacological actions similar to those of bradykinin in samples recovered from saline deposits injected into the dermis of sweating subjects. The failure to demonstrate sympathetic cholinergic fibers supplying human skin blood vessels offers inconclusive support of the bradykinin hypothesis. It represents only a part of a body of ignorance which includes lack of precise knowledge of the manner of termination of adrenergic fibers to skin vessels as well. In the hind legs of cats and dogs sympathetic cholinergic fibers were found to be distributed only to vessels of skeletal muscle (16).

(a) Effects of thermal sweating upon blood flow distribution in skin. The experiments reported in this section were designed to study changes in helium leak rate through the skin simultaneously with measurements of forearm blood flow, sweat rate and skin temperature.

Procedure:

Subjects were prepared for recording of blood flow from one forearm; sweat rate, skin temperature and the rate of leakage of helium from the skin were recorded from the opposite arm. The subjects rested on a chaise in a temperature-controlled room. The initial temperature of the room was 25° to 28°C. Breathing mixtures administered by face mask from demand regulators were air from the house supply or a mixture of 80 per cent helium and 20 per cent oxygen. In experiments using heat as the stimulus to sweating, subjects were equilibrated for 30 minutes at 25° to 28°C and then subjected for varying periods to ambient temperatures of 35° to 42°C. Our equipment did not provide control of humidity. On days when the relative humidity was below 50 per cent, it was necessary to heat a vessel of water in the temperature-controlled room in order to provoke sweating at desired rates. Unless otherwise specified, the temperatures given in the text or on illustrations are dry-bulb measurements. For some experiments continuous breathing of helium mixture occurred throughout the entire heating period; in others, the helium mixture was administered until the leak rate through the skin began to achieve plateau level -- usually about 15 minutes in young subjects.

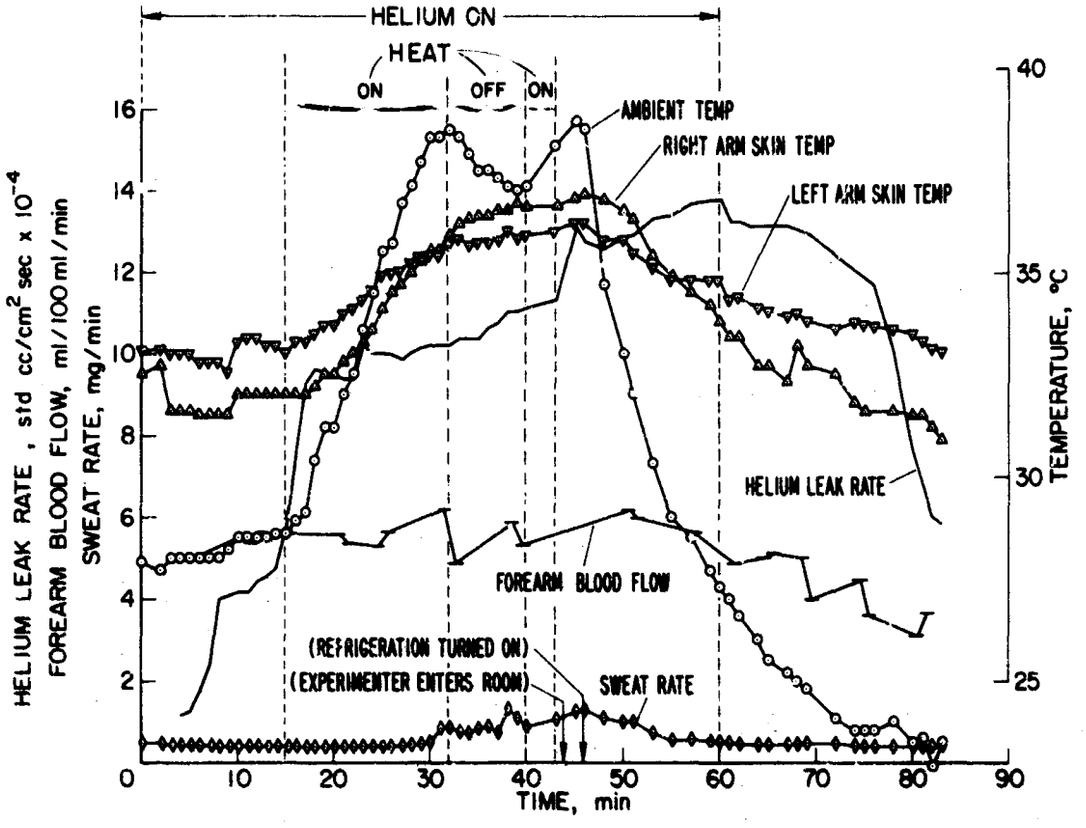

Figure 3-8 General features of the relationships of helium leak rate to blood flow changes and sweating during general body heating.

Results and Discussion:

The general features of the relationship of helium leak rate to blood flow changes and sweating during general body heating are shown in figure 3-8. The subject began breathing helium mixture at zero time. During the equilibration period forearm blood flow and skin temperatures were relatively stable. Helium leak rate had begun to level off, but it rose sharply with the onset of heating without any recorded change of forearm blood flow or sweat production. Skin temperature did not change appreciably during the rapid increase in helium leak rate. This pattern of events suggests that the number of open superficial capillaries increased and accomplished a redistribution of blood flow before the beginning of an actual increase in the rate of skin perfusion.

Sweating was detected only during the last four minutes of the initial heating period, but it continued to increase slightly during the 8 minute period during which the heat was turned off. Helium leak rate continued to increase roughly in proportion to the increase of skin temperature. The sharp peak of helium leakage appearing about 3 minutes after the end of the second heating period is probably part of the subject's "alerting" response evoked when the experimenter entered the room to turn on the refrigeration system.

When ambient temperature was decreased at 46 minutes, the usual decline of skin temperature began. Helium leak rates continued to rise slightly while sweat rates and forearm blood flow rates were decreasing. After the breathing mixture was switched from helium mixture to air at 60 minutes, helium leak rate from the skin remained nearly constant for the duration of pulmonary wash-out, about 7 to 8 minutes.

After prolonged inhalation of 80 per cent helium, the degree of saturation of body fluids in all compartments creates a large reservoir of helium, most of which is transported by the blood to the lungs where it is eliminated over a period of hours. The rate of elimination from the skin follows roughly the same general time course but is quantitatively much smaller. Both total skin blood flow and the distribution of that flow in the skin affect the rate of helium loss from the skin. When the number of open superficial skin capillaries is decreased by closure of precapillary sphincters, a part of the helium dissolved in skin water becomes isolated from exchange with the circulating blood, but it is still capable of diffusing through the skin surface. This isolation was partly

responsible for slowing the decline of helium leak rate for 16 minutes after the subject began to breathe air. Ambient temperature was 27.3°C at the time the helium mixture was discontinued. Forearm skin temperatures were 34.8° and 33.8°C.

Some features of the saturation phenomenon have been studied and will be discussed in later sections of this report. A rigorous characterization of the kinetics of saturation and desaturation has not been completed owing to termination of the contract. Necessary measurements of helium in arterial and venous blood from deep and superficial cutaneous veins had to be postponed. The development of suitable sample introduction systems capable of yielding direct measurements of helium in blood and other fluids has been accomplished: their use in the solution of this problem has not.

(b) Effect of carbon dioxide inhalation upon helium leak rate and sweating in subjects resting at sub-sweating skin temperatures. Interpretation of blood flow measurements and rates of helium leakage from the skin when sweating is evoked by general body heating is complicated by interaction of central, spinal reflex, and possibly blood-borne humoral factors acting on the sweat glands and blood vessels together or in unknown sequence. Increasing ambient temperature contributes sensory input from the skin to the central temperature regulating center and also stimulates metabolic activity in skin cells. How much of the increased skin blood flow measurable in heated subjects is attributable to autoregulatory changes in vascular resistance is not known. The role of autoregulation in modifying the distribution of blood flow within the skin is no better understood. We have attempted to separate sweat secretion as a factor possibly influencing skin blood flow from the usual complications introduced when heat is used as the stimulus. We have administered acetyl-beta-methyl choline (mecholyl) or pilocarpine nitrate by ion transfer into the skin of subjects maintained in a cool environment (28). Sweat gland activity can be sustained at high levels by this means. Unfortunately both drugs are also local vasodilators. Changes in blood flow that occurred with drug-induced sweating could not be identified as secondary to the sweat gland response or as part of a direct response to the drug.

Bullard (55) has reported that sweat gland activity was stimulated in subjects maintained at temperatures below sweating threshold during brief periods when they were breathing 7 per cent carbon dioxide. In the hope of being able to separate the effects of sweat gland activity upon

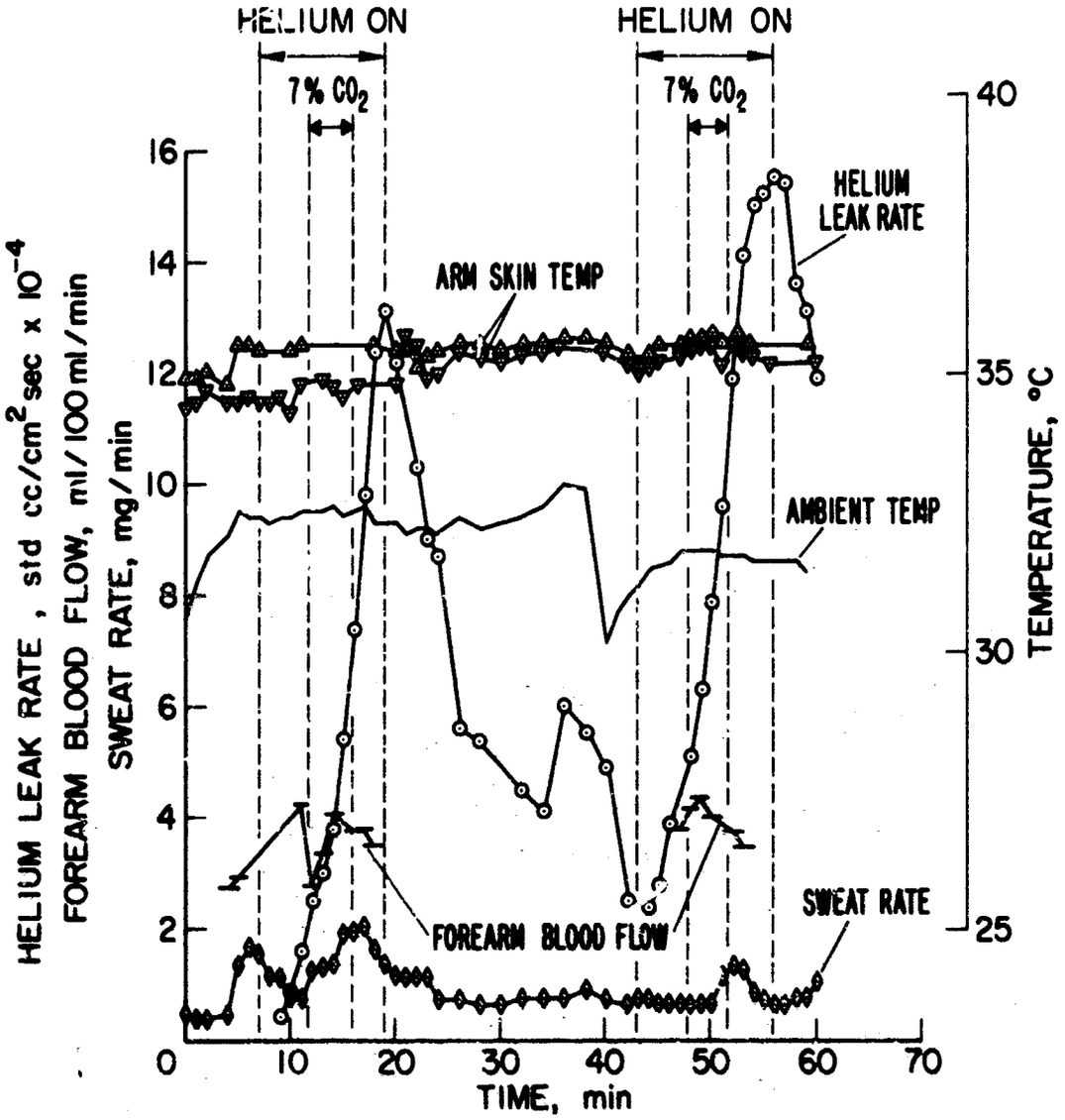

Figure 3-9 Sweating and increased helium leak rate induced in subject at sub-sweating threshold temperature by inhalation of CO_2.

the skin circulation from the neurovascular events usually associated with sweating, we have explored the usefulness of Bullard's procedure.

Two new gas mixtures were obtained with 73 per cent helium and 20 per cent oxygen in each. One of the size "G" cylinders contained 7 per cent nitrogen and the other contained 7 per cent carbon dioxide. The two cylinders were connected through a dual manifold with valves permitting the helium mixture demand valve to be supplied from either tank by switching-valves outside the temperature-controlled room. The subjects were prepared as described previously for recording of helium leak rate, skin temperature, sweat rate and forearm blood flow. With the subject at rest on a chaise and the breathing mask not yet applied, the room temperature was increased sufficiently to elicit episodes of sweating. The temperature was then lowered gradually until sweating stopped or occurred in widely separated bursts. The breathing mask was adjusted, the subject was allowed to breathe air for 3 to 5 minutes and was then switched to the helium-nitrogen-oxygen mixture. At 5 minutes after the beginning of breathing the initial helium mixture, the helium supply was switched to the tank containing 7 per cent carbon dioxide in place of nitrogen for four minutes and then was switched back to the initial helium mixture. Figure 3-9 shows data obtained from two such periods. The first of the two periods illustrates a set of measurements made under conditions that did not meet the criterion of absence of sweating prior to the administration of CO_2. Helium leakage and blood flow increases accompanied a burst of sweating that began before the gas mixtures were switched. The second period was carried out after lowering the ambient and skin temperatures slightly. In this example, sweating was delayed about 2.5 minutes, but the steepening of the rise of helium leak rate began promptly with the beginning of CO_2 breathing and continued to be rapid for the four-minute period. After the mixtures were changed, the rate of increasing helium leakage fell off, even though the same concentration of helium was being administered for about 4.5 minutes after the cessation of CO_2 inhalation. In both examples, sweating began during the last half of the period of CO_2 breathing and was preceded by a large increase of helium leakage, with no clear rise of skin temperature. The peak of sweating occurred during a decline of forearm blood flow. Thus, in the case of sweating induced by CO_2 inhalation, large increases of helium leak rate occurred without evidence of increased skin blood flow and must be presumed to indicate opening

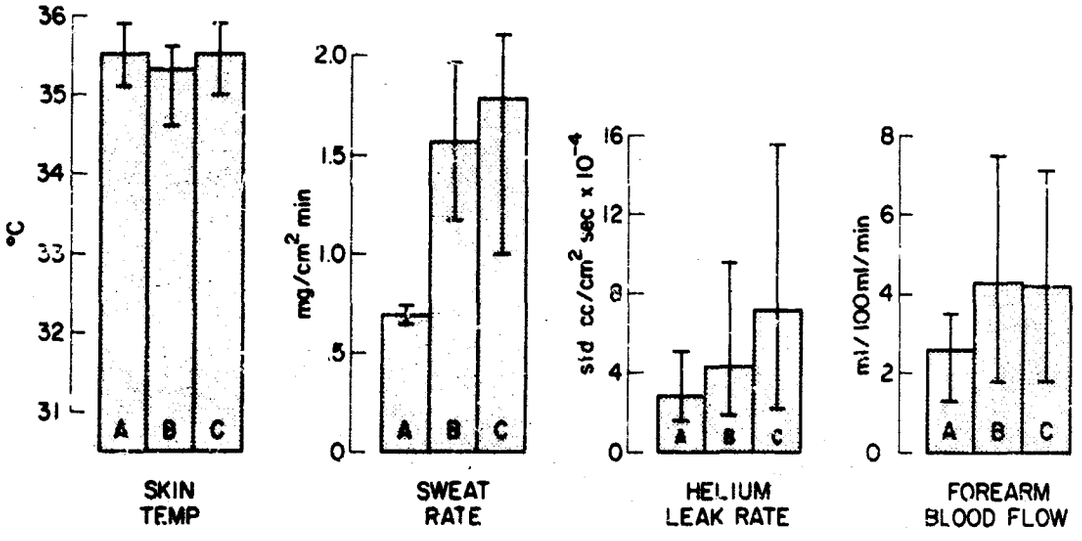

Figure 3-10 Summarized data from four experiments showing effects of CO_2 inhalation on sweating and helium leak rate. A = control period; B = measurements during CO_2 breathing; C = post-CO_2 maximum.

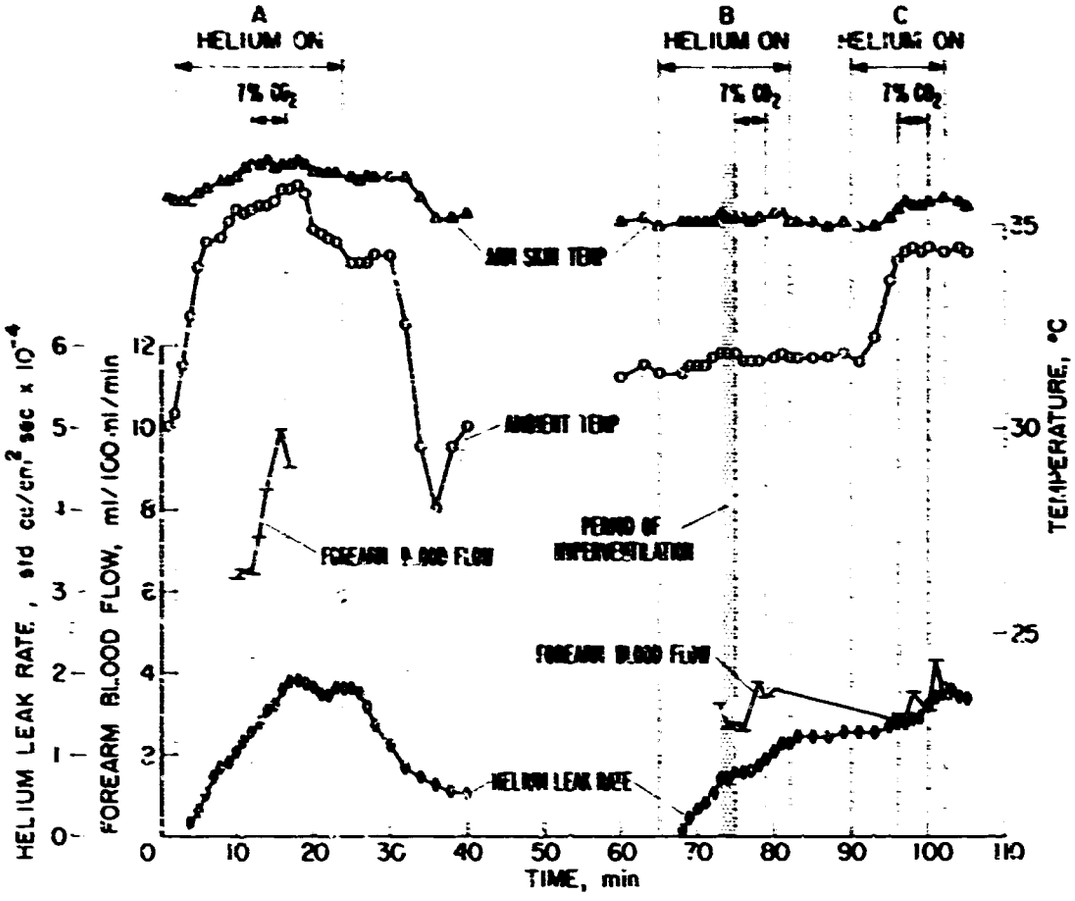

Figure 3-11 Effects of breathing carbon dioxide and of temperature upon forearm blood flow, skin temperature and helium leak rate. A. measurements during increased ambient temperature from 30.0° to 35.9°C; B. measurements at ambient temperature slightly less than 32°C and skin temperature stable at 35°C. Subject hyperventilated for 2 minutes before breathing CO_2; C. skin temperature raised to 35.5°C before starting CO_2 breathing.

of a larger number of superficial skin capillaries before there was evidence of dilatation of resistance vessels in the skin blood supply.

Figure 3-10 presents summarized data from 4 experiments similar to those shown in figure 3-9. Each bar in the histograms has the top drawn at the mean value of each set of measurements; the vertical lines show the extent and distribution of the ranges. The large interindividual variations in sweat rate, helium leak rate and forearm blood flow indicate that many more experiments must be done before firm general conclusions can be drawn.

Figure 3-11 illustrates three successive tests of helium leak rate and forearm blood flow: (A) to examine the combined effects of general body heating and CO_2 inhalation; (B) to test the effects of hyperventilation prior to CO_2 inhalation during maintenance at skin temperature 0.5°C below the previously established sweating threshold; and (C) to test the effect of rapidly increasing skin temperature to the sweating threshold. Forearm blood flow, skin temperature and helium leak rate were recorded. Sweat rate could not be measured because of equipment failure.

In part (A), room temperature was rapidly increased from 30°C to 35.9°. The breathing mixture was changed from air to helium-nitrogen-oxygen at the time heating was started. Visible sweating was noted when the skin temperature reached 35.8°C. The breathing mixture was switched to helium-carbon dioxide-oxygen 10 minutes after the beginning of helium breathing. A substantial increase of forearm blood flow occurred during the four-minute period of CO_2 inhalation without any detectable change in the slope of the rising rate of helium leakage until 30 seconds after the end of the CO_2 inhalation. The flattening of the helium leak rate and the slight decrease during the remainder of the period of helium breathing coincided with a decrease of almost 2°C in room temperature and about 0.5°C in arm skin temperature.

Part (B) was carried out after room temperature had been reduced to slightly less than 32°C and skin temperature was relatively stable at 35.0 minus 0.1 and plus 0.2°C. Forearm blood flow measurements were started 7 minutes after the beginning of helium breathing and continued for 7 minutes. The subject then hyperventilated near his maximum breathing capacity for 2 minutes before the change of breathing mixture to administer carbon dioxide. During the

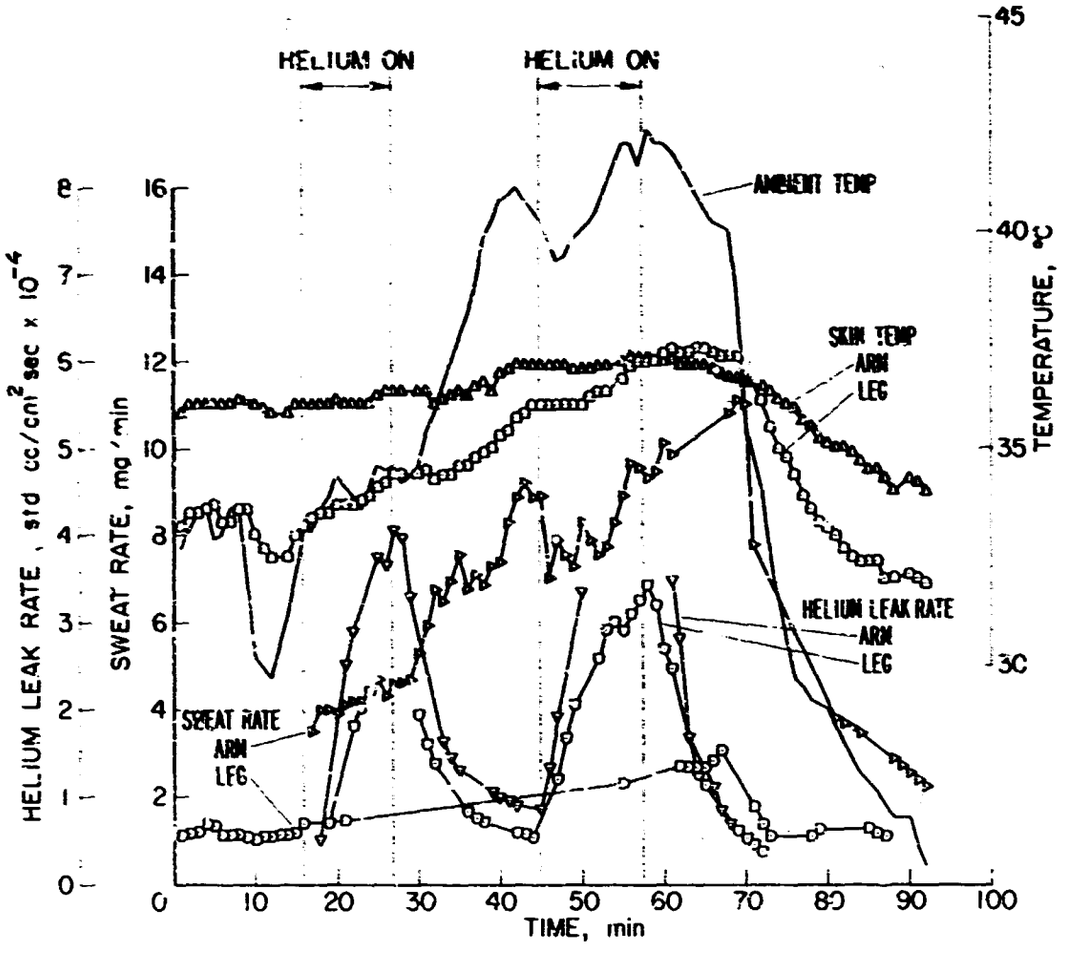

Figure 3-12 Simultaneous measurement of forearm and leg sweat rates and skin temperatures and alternate sampling of helium leak rate from arm and leg skin during general body heating.

2 minutes of hyperventilation, the forearm blood flow declined slightly, and the rising rate of helium leakage became virtually flat. In the last 1.5 minutes of CO_2 inhalation, both forearm blood flow and helium leak rate began to increase slightly. The decreased forearm blood flow produced by hyperventilation did not involve sufficient cutaneous vasoconstriction to decrease skin temperature significantly. The combined effects of decreased temperature and lowered pCO_2 reduced the superficial skin capillary exchange surface enough to interrupt the rising leak rate of helium.

In part (C), room temperature was increased until skin temperature rose to 35.5°C. There was a moderate increase in both blood flow and helium leak rate during carbon dioxide inhalation and a steeper rise in the brief period following. The augmentation of helium leak rate during inhalation of CO_2 was much smaller than had been obtained on the same subject in other experiments similar in procedure to those summarized in figure 3-10 with the skin temperature at 35.5°C but without antecedent hyperventilation. It may be that some of the effects of the hyperventilation persisted and were responsible for the diminished response.

(c) Comparison of some regional differences in skin temperature, sweat rate and helium leak rate. Regional differences in skin temperature, thermal gradients and heat loss rate have been extensively reported (56). Adamczyk et al (44) recorded helium leakage from 15 skin areas with a mass spectrometer and found a 6-fold difference between the slowest and fastest leak rates. These investigators made no measurements of skin temperature or blood flow nor did their report state what posture was assumed by the subjects during the measurements.

We have reported some data (see Part 2, 1 (c)) on differences in the relationship of blood flow to helium leakage that were obtained from simultaneous measurements on fingers and forearm skin. Figure 3-12 shows data obtained from simultaneous measurements of forearm and leg sweat rates and skin temperatures and alternate sampling of helium leak rate from arm and leg skin during general body heating by increasing the ambient temperature from 33 to 42.3°C. The subject was seated, with legs extended, on a chaise and breathed air during equilibration at about 32 to 33°C. Helium leak rates were measured during about 10 minutes of breathing of the He/O_2 mixture. The breathing mixture was switched to air and the room was rapidly heated to elicit sweating. The ambient temperature

was 41°C when the arm sweat rate had reached a level approximately double that during the initial measurement of helium leak rate. Helium leak rate was again measured as room temperature rose irregularly to 42.4°C. The findings of principal interest were that helium leak rate, skin temperature and sweat rate were higher in the forearm than in the leg and that the relationship held during vigorous sweating. This observation is in accord with differences reported for blood flow (7), density of visible capillary loops (9) and eccrine sweat glands (58) in the two regions studied.

(d) Effect of atropine on skin blood flow. Atropine administered by intra-arterial injection (54) or by ion transfer (11) blocks sweat secretion from the treated areas in subjects exposed to general body heating. The increase of forearm blood flow usually associated with sweating was found to be only temporarily suppressed. Shepherd (58) pointed out that the eventual increase of blood flow in the atropinized forearm did not indicate waning effectiveness of the atropine, since the vessels were still resistant to the effects of injected acetylcholine, and the skin did not sweat.

We have made use of atropine administered by ion transfer to produce a dissociation between the process of sweat secretion and the attendant changes in skin blood flow for the purpose of studying the role of sweat gland activity in the increased rate of helium leakage during general body heating.

Procedure:

Administration of atropine or sodium chloride. Subjects were prepared for ion transfer of atropine for sweat gland suppression and sodium chloride solution to the opposite arm for the control by close clipping of all hair from a 10-cm band in the mid-portion of each forearm. A 0.01 per cent solution of atropine sulfate in distilled water was used to saturate a piece of four-ply surgical gauze 11 cm wide by 34 cm long. The clipped skin area was scrubbed thoroughly with soap and warm water, rinsed and dried. The skin was then wiped with acetone sponges and dried. The saturated gauze was squeezed drip-free and applied smoothly about the mid-portion of one forearm. The positive electrode was made from 30-mesh stainless steel screen, bias-cut to a width of 9 cm and a length of 34 cm. The electrode was applied over the gauze pad so as to avoid any

direct contact of metal with skin, and was held in place by a 40-cm length of one-inch surgical drainage tubing. Because of the bias cut, the width of the electrode was less than 8 cm after it was applied. The negative electrode was a Crooke's metal strip immersed in a 1-liter beaker partly filled with 0.01 per cent NaCl solution. Contact of the negative electrode with the skin was provided by having the subject immerse the hand of the extremity being treated so that the level of saline solution reached as far as the wrist.

The power supply for the ion transfer was a constant current source, with solid-state circuitry and manual controls. The unit was transformer-isolated for protection against shock hazard. Electrical charge transfer was limited to 54 millicoulombs per square centimeter, determined by time of known current flow. The current density used was not over 0.15 ma/cm^2. The areas of skin treated in each forearm of the three subjects used for these experiments were 192, 205 and 232 cm^2. In each experiment, one arm of the subject was used for treatment with atropine; the other arm was similarly treated by ion transfer using 0.01 per cent sodium chloride solution to saturate the gauze pad. In six experiments each arm of each subject was studied once after atropine treatment and once after treatment with sodium chloride.

Preparation for measurements. The temperature of the controlled room was set at a moderately warm level (35.5° to 38.0°C). A one-hour equilibration period of the subject started 15 to 30 minutes after completion of the ion transfer procedures. Mercury-in-silastic strain gauges, capsules for sampling sweat rate and helium leak rate, and thermistors were placed in symmetrical positions on the two forearms within the treated areas.

Recording of temperatures from each of the thermistors was repeated once each minute from zero time by means of an electric timing switch. Sweat rate was recorded continuously from the output of a resistance hygrometer (33). Helium leak rate was recorded alternately from the two forearms every two minutes. Blood flow rates were measured in both forearms simultaneously at intervals throughout the heat exposure period. Seven to twelve venous collections were made for each measuring period and averaged to obtain mean values for plotting.

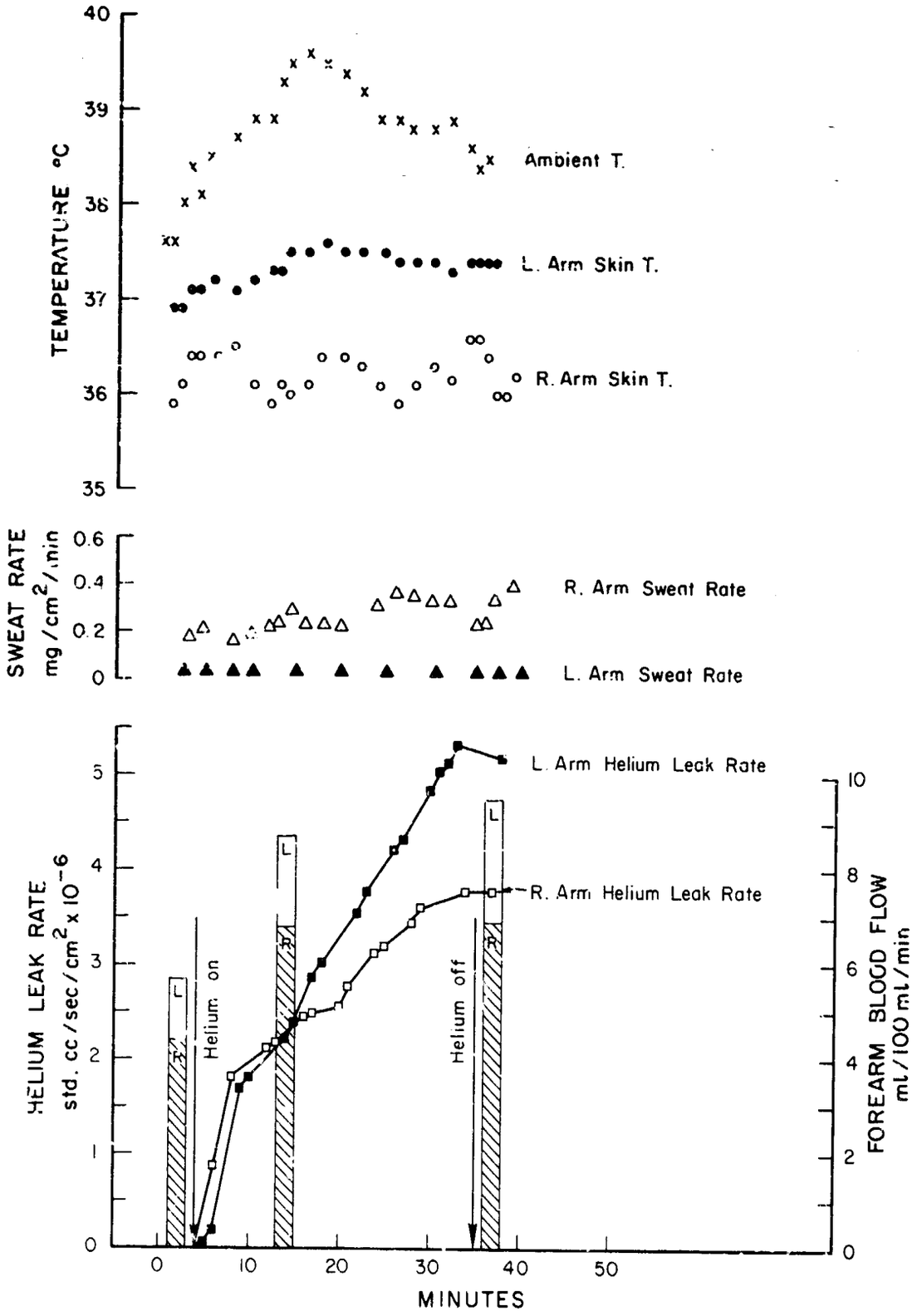

Figure 3-13 Increase of skin temperature, forearm blood flow and helium leak rate during general body heating after treating forearm skin with atropine by ion transfer.

Results and Discussion:

Figure 3-13 shows data from a representative experiment. The subject's left forearm had been treated with atropine and the right arm with sodium by ion transfer 90 minutes before zero time. Effective suppression of sweating in the atropinized arm is illustrated by the near-zero sweat rate shown in the left arm while the right arm was sweating at about 0.2 mg/cm^2/min. The skin temperature of the left arm remained higher than that of the right arm throughout the measurement period. Blood flow in the left arm, shown as the total height of the bars, was 1.5 to 2 ml/100 ml/minute above that in the right forearm, shown as the shaded part of the bars.

The helium leak rate from the skin of the right forearm was of the usual magnitude and displayed a somewhat delayed but definite trend toward plateau toward the end of the helium breathing period. The helium leak rate from the left forearm was slightly below that of the right arm for the first eight minutes but maintained a nearly linear upward course for the remaining 15 minutes of helium breathing.

Although flushing of the skin, particularly in the blush areas, is a usual phenomenon observed in individuals who have received toxic doses of atropine, it is seen only occasionally in those who have received ordinary clinical doses (57). We have noted slight redness of the skin, persisting for about 20 minutes after administration of atropine by ion transfer. However, similar color changes were seen in the contralateral arm following ion transfer with sodium chloride as the electrolyte. We have considered that the transient redness of the skin is probably a non-specific effect of current flow and not an example of "atropine flush" sometimes associated with toxic doses of atropine administered orally or parenterally.

Skin temperature, helium leak rate and forearm blood flow were higher in the atropinized arm than in the control arm in all six of our experiments. The data reported by Roddie et al show that forearm blood flow in the atropinized arm lagged behind that in the control arm during most of the period of general body heating. Skin temperatures and helium leak rates were not measured. Roddie and his associates administered atropine by intra-arterial injection, but there is no reason to suppose that the route of administration was responsible for the different findings. Blood flow was measured by means of a water-filled

plethysmograph that covered 7-cm segments of each forearm. The temperature of the water was maintained at 34°C throughout the experiment. Our plethysmograph was a mercury-in-silastic strain gauge covering a band of skin about two millimeters wide. Both the skin and the gauge were exposed to ambient temperature. The temperature difference observed in the two forearms was possibly the result of evaporation of sweat from the control arm and the absence of sweating and evaporation on the atropinized arm. Since the temperature of the skin in the atropinized arm was persistently elevated above that of the control arm, there was a larger thermal stimulus in the atropinized arm to metabolism of skin cells and auto-regulatory inhibition of precapillary sphincters, opening of more superficial capillaries and a drop in post-arteriolar resistance to blood flow. The data provide no way of identifying the separate roles of segmental reflexes and myogenic autoregulation as factors in the vasodilatation occurring in the atropinized forearm.

The trend of helium leak rate from the control forearm toward plateau and the absence of plateau in the helium leak rate from the atropinized forearm support the hypothesis that capillary area available for diffusion was greater in the atropinized forearm. With respect to the role of sweat gland activity in the increased rate of helium leakage during general body heating, the data show only that absence of sweat gland activity does not diminish the rate of helium leakage from skin if other conditions of the experiment permit skin blood flow to rise.

(e) Effects of superficial capillary blood flow upon helium leak rate. In order to assess the effects of increasing the number of open capillaries in the superficial layers of the skin upon skin temperature, forearm blood flow and helium leak rate, two methods were used. In the first of these, isopropyl-nor-epinephrine (isoproterenol), as the racemic mixture, was administered by the same procedures as those used for atropine. Isoproterenol has been reported to relax forearm resistance vessels, presumably through its action on β-adrenergic receptors in arteriolar smooth muscle, without any significant effect on the smooth muscle of precapillary sphincters (59). The authors reporting this observation administered the drug by close intra-arterial injection and based their conclusion upon measurements of capillary filtration capacity. By administering isoproterenol directly into the skin, we hoped to produce a similar differential effect upon skin arterioles and capillaries: an increase of skin blood flow with minimal increase in the capillary area available for exchange.

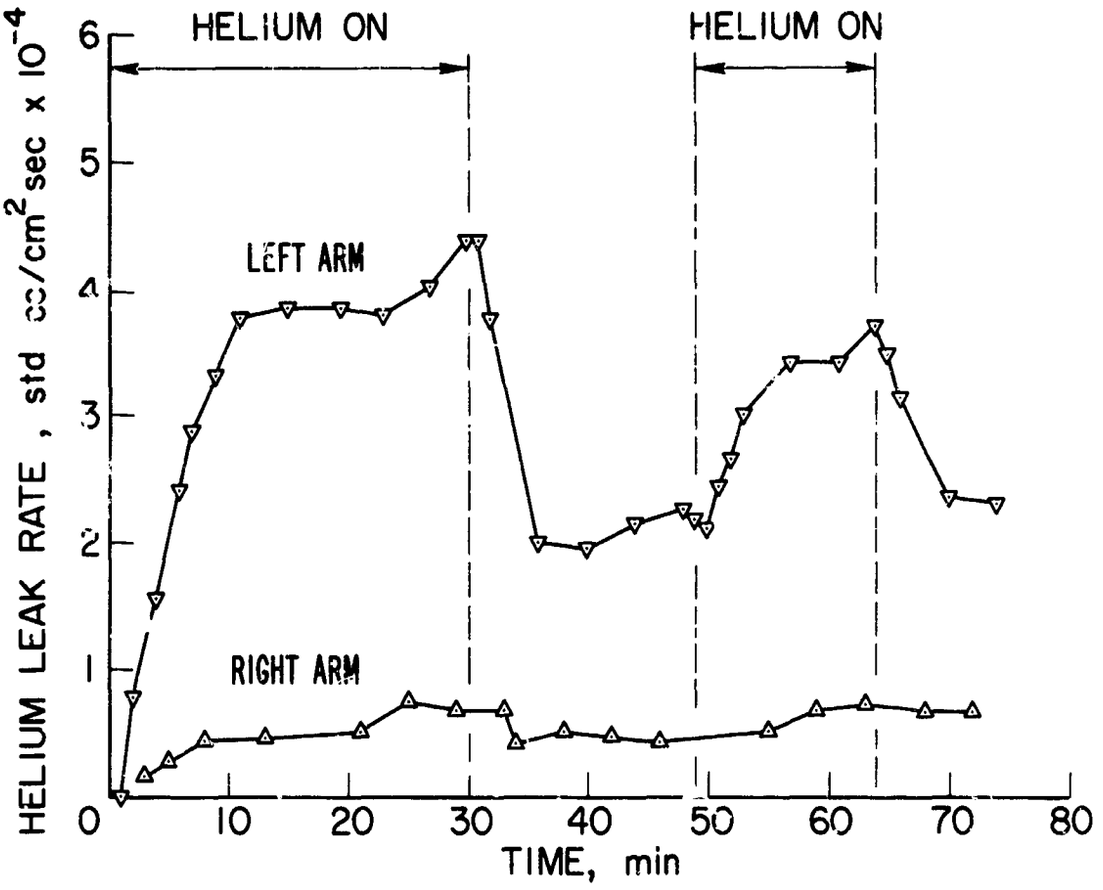

Figure 3-14 Effect of isoproterenol on helium leak rate. Measurements made 30 minutes after ion transfer of a 0.1% solution for 15 seconds at a current density of 0.3 ma/cm^2 to 19.6 cm^2 of skin on the volar surface of the left forearm.

In the second group of experiments, local heating of one forearm was used to produce increased skin blood flow secondary to increased filling of superficial capillaries. In both groups of experiments, the subjects rested at a comfortable ambient temperature. Neither procedure produced sweating, and therefore sweat rates were not recorded. All other measurements were the same as those described for the experiments with atropine.

The intent was to eliminate as far as possible the contribution of central (hypothalamic) participation in the responses observed. Reflexes of the Gibbon-Landis type, causing changes in blood flow in the control arm resulting from temperature changes in the arm treated with isoproterenol or from the locally heated arm, could not be excluded.

Effects of isoproterenol. Preliminary tests were conducted to establish required dosage, necessary duration of current flow for ion transfer and presence or absence of signs of systemic effects. Patch tests were made with 2 ml of a 0.1 per cent solution of isoproterenol on a 2" x 2" gauze sponge kept for 18 hours under an occlusive dressing on the volar surface of one forearm. The test area was examined at 1 hour, 3 hours and 18 hours after application. At no time was there evidence of blanching or redness.

Ion transfer of a 0.1 per cent solution of isoproterenol to a circular area of the volar surface of the forearm at a current density of 0.3 ma/cm^2 for 1 minute was carried out with the positive lead from the current source attached to a 19.6-cm^2 circular electrode. The treated area had a mottled appearance (blanching and mild redness) when first examined. Within ten minutes blanched areas had become red and slightly warmer to the touch than the surrounding skin. The redness did not begin to fade until over 8 hours after the ion transfer. Subsequent tests at the same solution strength but with 15- and 30-second current flows at 0.3 ma/cm^2 indicated that adequate dosage for experiments lasting 4 hours could be administered with current flow lasting for 30 seconds.

Figure 3-14 shows data on helium leak rates in the normal arm (lower curve) and the arm treated with isoproterenol 0.1 per cent solution for 15 seconds at 0.3 ma/cm^2 over a circular area of 19.6 cm^2 on the volar surface of the forearm. The subject was a 61-year-old man resting on a chaise at an ambient temperature of 25° to 26°C. The helium leak rate in the isoproterenol-treated

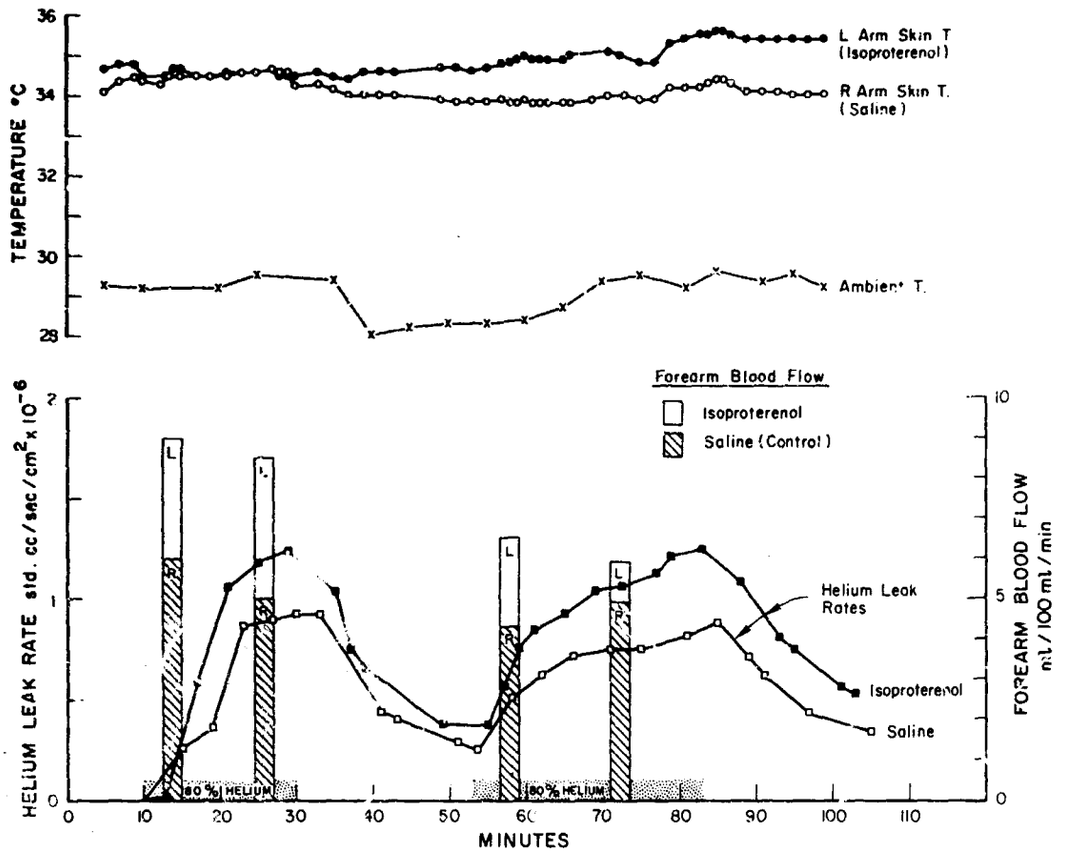

Figure 3-15 Effects of isoproterenol on forearm blood flow, helium leak rate and skin temperature.

arm is as high as that observed during vigorous sweating in the same subject.

Tests with the 11-cm x 34-cm gauze electrode were also carried out to determine whether the dosages given over a larger skin area had detectable systemic effects. It was found that current densities that were well tolerated when applied through the small circular electrode were intolerably painful when the larger electrode was used. The current density that was reasonably well tolerated was found to be 0.075 ma/cm^2. Calculated time for the 23.1 ma required to equal the 30-second dosage with a 0.2 per cent solution of the drug was 80 seconds. At this dosage systemic effects of a subjective nature were minimal. Heart rate at 10 minutes after ion transfer was found to have increased from 80 at the beginning of the test to 92 beats per minute, with return to 80 per minute at 50 minutes.

Figure 3-15 shows complete data on ambient and skin temperatures, helium leak rates and forearm blood flow measurements after ion transfer of isoproterenol and sodium into the skin of the forearms by means of the large gauze electrodes. Current density, solution concentrations, total current, and time were the same as those described above for the large electrodes. Zero time on the figure marks 126 minutes after the second ion transfer was completed. Skin temperature, forearm blood flow and helium leak rates are all higher in the arm treated with isoproterenol.

The factors governing helium leak rate are clearly different from those dominating the pattern during exposure to heat after treatment with atropine. The levels reached by the helium leak rates are lower than those reached in the atropine-treated forearms during general body heating, and the dynamics of saturation are shown in both the control and the isoproterenol-treated arm by the clear development of the plateau phase of helium leakage during both episodes of helium breathing.

The differences in blood flow and skin temperature in the two arms are consistent with the reported action of isoproterenol: relaxation of resistance vessels with little effect on precapillary sphincters. It is safe to assume that the first encounter of isoproterenol with vascular smooth muscle occurred at the precapillary sphincters guarding the capillary loops that extend into the dermal pegs. If these sphincters had been relaxed by the drug, the increase of capillary area available for diffusion of

helium from the blood would have been markedly increased at the minimum depth below the skin surface. Leakage of helium through the skin under these circumstances would become, as nearly as possible, limited only by blood flow. Instead, the helium leak rate curves in both arms show the development of plateau phase at about the same time after the beginning of helium breathing. Relaxation of smooth muscle in resistance vessels of the arm treated with isoproterenol accounts for the differences in forearm blood flow and the increased delivery of helium to the treated area. Skin temperature was slightly higher in the arm treated with isoproterenol. A modest increase in the number of open superficial skin capillaries might have occurred, but the transfer of the major site of resistance to flow produced by the action of isoproterenol on the arteriolar smooth muscle placed the precapillary sphincters under increased tension. This stimulus to myogenic autoregulation limited the degree of participation of the superficial capillaries as conduits for the increased blood flow (60).

Effects of local heating. Local application of heat to the skin has been shown to increase skin blood flow. Segmental suppression of vasoconstrictor tone is probably involved as part of the mechanism of relaxation of resistance vessels when nerve function is intact. Sympathectomy, traumatic denervation, and blockage of cutaneous nerves does not abolish the vasodilator response to local heating (61). The process of vasodilatation produced by local heating is believed to begin as a relaxation of precapillary sphincters (metabolic autoregulation) followed by a succession of myogenic responses to decreased wall tension in resistance vessels (62).

Local heating of one forearm in subjects resting at a comfortable ambient temperature was employed to produce maximal opening of the superficial capillaries in the skin. The aim of this procedure was to reduce to a minimum the distance of helium diffusion and to increase to a maximum the capillary area available for diffusion.

A second procedure was employed in this group of experiments to demonstrate the effect of a sudden increase in the partial pressure of helium in arterial blood upon the plateau phase of helium leak rate. A breathing mixture containing 20 per cent helium, 20 per cent oxygen, and 60 per cent nitrogen was obtained. Size "G" cylinders of this mixture and of the usual 80 per cent helium and 20 per cent oxygen were connected to the dual manifold and

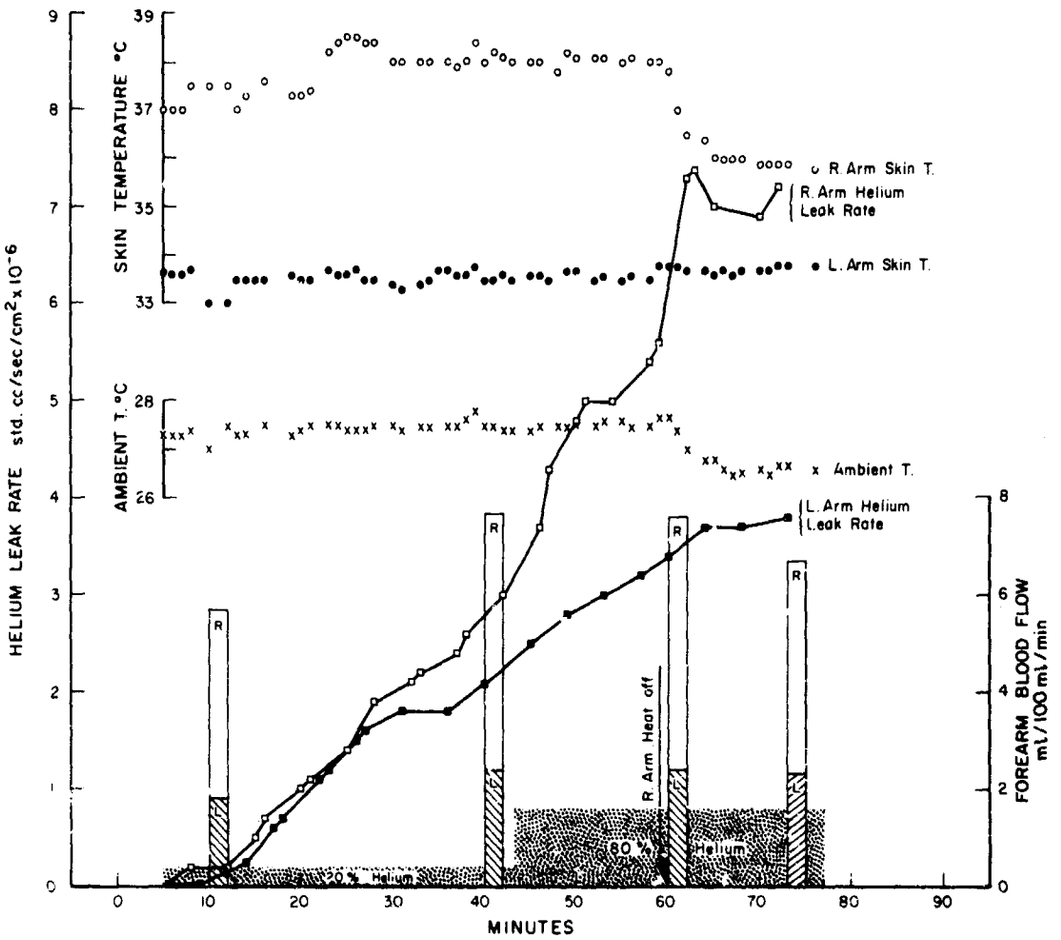

Figure 3-16 Skin temperatures and forearm blood flow rates during local heating of the left arm and hand. Helium leak rates were measured during breathing of 20% helium and then 80% helium mixtures.

valve system. Either of the two mixtures could be delivered at will to the demand valve serving as the face mask supply.

Ambient temperature was controlled at a comfortable level (22.5° to 27.5°C). Both arms of the subject were prepared for measurement of forearm blood flow, skin temperature and helium leak rate. The arm to be heated was enclosed in a polyethylene tube, about 20 cm in diameter when inflated into cylindrical form. About 60 cm of the tube were used to enclose the hand and forearm by securing the tubing loosely above the elbow and connecting the other end beyond the hand to the hose from an ordinary hair dryer. The hair dryer was controlled manually through a variable transformer to maintain the skin temperature of the heated arm in the range of 37.5° to 40°C.

Figure 3-16 presents the data from a representative experiment in which heat had been applied to the right arm for the last 10 minutes of a 30-minute equilibration period before zero time. When recording was begun at 5 minutes, the skin temperature in the heated arm was 3.4°C above that in the control arm. Within the period when the breathing mixture contained only 20 per cent helium, the helium leak rate from the control arm developed a clear plateau phase, but that from the heated arm continued to rise as the skin temperature increased by a little over 1 degree.

When the breathing mixture was switched to 80 per cent helium, the helium leak rate increased in both arms. The difference in helium leak rate increased progressively until the local heating was discontinued at 60 minutes. The decreased helium leak rate in the right arm and the flattening of the curve from the left arm in the last 15 minutes probably reflect the influence of the decreased skin temperature in the right arm and the decline of ambient temperature. Hence, these are not examples of development of plateau in the usual sense.

Forearm blood flow levels were approximately three times as high in the right arm as in the left, in the last 20 minutes before heating was stopped, and the respective levels did not change during the transition from 20 per cent helium to 80 per cent helium breathing. In view of the relatively stable skin temperatures in the two arms, no factor other than increasing partial pressure of helium in arterial blood could be demonstrated to account for the rising helium leak rates in the two forearms. The expanding difference between the leak rates

recorded from the control arm and the heated arm from the onset of breathing 80 per cent helium depends partly upon the difference in blood flow and partly upon the difference in blood flow distribution in the skin of the two arms.

It is assumed that the entire difference of blood flows in the heated and unheated forearms can be attributed to the larger volume of blood passing through skin vessels of the heated arm. The ratio of the virtual volume of blood required to deliver the amount of helium leaking through the skin to the measured difference in blood flow should express the relative size of the "effective" blood flow. The concept of a virtual volume of blood required to supply the constituent exchanged is analogous to that implicit in the measurements of renal clearance. It shares with the clearance measurement the requirement that arterial plasma or blood must be used to obtain the value of P or B in the clearance equation:

$$C_x = \frac{U_x V}{P_x \text{ (or) } B_x},$$

where C_x = virtual volume of plasma or blood "cleared" of x per minute (ml/min)
U_x = concentration of x in urine (mg/ml)
V^x = urine volume (ml/min)
P_x or B_x = plasma or blood concentration of x (mg/ml).

The analogous relationship for helium leak rate through the skin would be:

$$C_{He} = \frac{jHe}{A_{He}}$$

where C_{He} = virtual volume of blood cleared of helium (ml/min)
jHe = helium leak rate from the skin covering 100 ml of forearm (ml/min)
A_{He} = helium concentration in arterial blood (ml He/ml blood)

In the absence of measured concentrations of helium in arterial blood, it is possible to make reasonable estimations of arterial helium concentrations to provide an example. For inert gases, equilibrium concentrations are related by pressure and solubility. In the calculations of arterial helium concentrations for Table 3-1, a barometric pressure of 760 mm Hg was assumed. Alveolar gas partial pressures

used to solve for final pHe and arterial helium concentration were: pO_2 = 100 mm Hg; water vapor at 37°C = 47 mm Hg; pCO_2 = 40 mm Hg. The value used for the solubility of helium in blood was 0.0088 ml He/ml blood, obtained from standard tables (41). Differences in values for helium leak rates and blood flows were obtained from data of the experiment shown in figure 3-16. The values for C_{He} were calculated from the helium leak rate differences corrected to amounts of helium per minute leaking through an area of skin equal to that covering 100 ml of forearm (48.1 cm^2 for the dimensions of our subject).

Table 3-1

Helium leak rates expressed as "clearances" and related to forearm skin blood flow during local heating of one arm.

1. Time	2. Helium leak rate differences (Right - Left)	3. Forearm blood flow differences (Right - Left)	4. C_{He} Right Arm	5. Ratio $\frac{C_{He}}{\Delta \text{Blood flow}}$
min	$cc/cm^2/sec \times 10^{-6}$	ml/100ml/min	$\frac{ml/min}{48.1\ cm^2}$	
35*	0.55	5.25	1.1	0.22
55	2.00	5.30	0.87	0.16
59	2.25	5.20	0.98	0.18

* Sampled during breathing of 20 per cent helium; data at 55 and 59 minutes during breathing of 80 per cent helium.

Verification of completeness of pulmonary wash-out, alveolar-arterial equilibrium of helium and concentrations of helium in deep and superficial cutaneous veins must await appropriate measurements in blood sampled in similar experiments. The calculations based upon assumed arterial helium concentrations and commonly accepted values for the partial pressures of water vapor are hypothetical, but they do suggest a means by which helium leak rate may be used to identify and quantitate redistributions of blood flow in the skin.

Klocke et al (53) concluded that transport of gases through the skin was a diffusion-limited rather than a perfusion-limited process. In a diffusion-limited situation, gas transport was expressed by the following equation:

$$\dot{V} = K \frac{A}{h} \cdot \frac{\alpha}{\sqrt{MW}}$$

where K = a constant
A = area of the diffusion surface (effective capillary surface)
h = the thickness through which the gas must diffuse
$\dot{V}$ = volume of gas transferred through the skin per unit time
α = the solubility of the gas
MW = molecular weight of the gas

Since $\dot{V}$ is a measured variable and K, α and MW do not change in the course of an experiment, variations in $\dot{V}$ are attributable only to changes in effective capillary surface area and differences in depth of capillaries from the skin surface. Comparison of helium leak rates and blood flow rates in locally heated forearms with the control arm at a comfortable temperature show that the increasing magnitude of $\dot{V}$ from the heated arm must be the result of increasing values of A/h. To the extent that smaller values of h participated, the appropriate interpretation would be that the opened capillaries were superficial rather than deep.

In a normally functioning circulatory system, there is no way that the opening of large numbers of additional capillaries can occur without evoking the proximal spread of relaxation of smooth muscles of the resistance vessels supplying those capillaries: an increase of minute volume blood flow is the inevitable consequence as long as perfusion pressure is adequate.

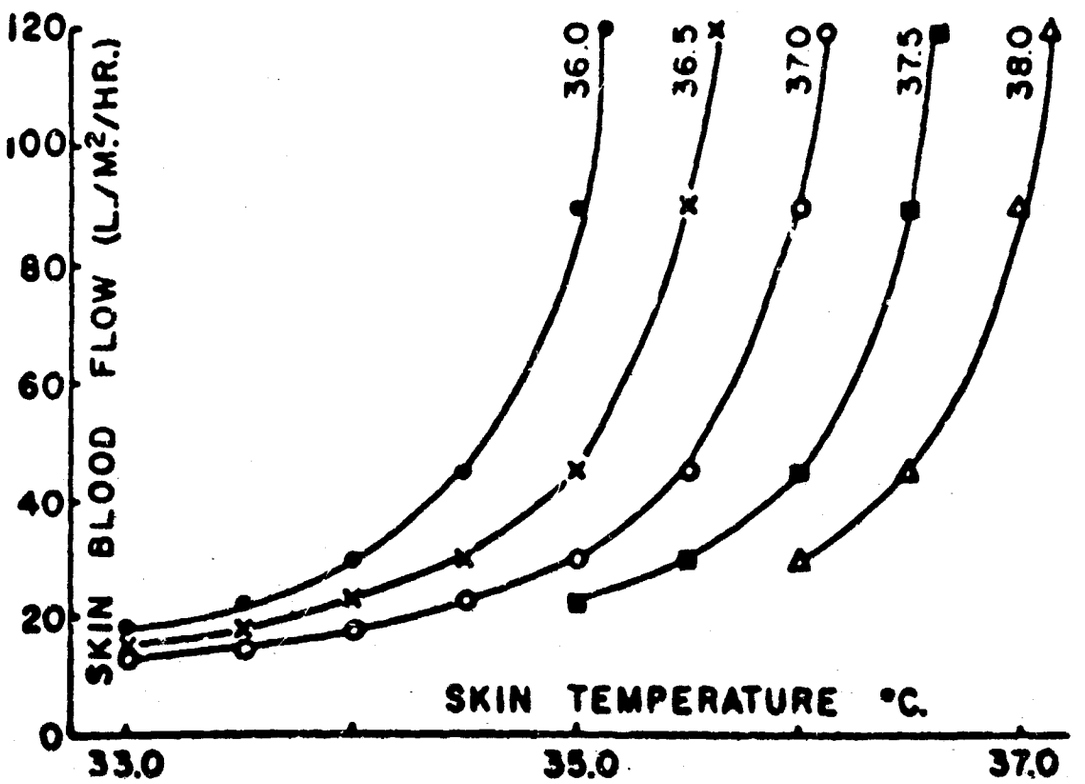

Figure 4-1 Theoretical relations of cutaneous blood flow and temperature at five different levels of core temperature. Blood flow calculated from assumed convective heat transfer. (From Senay, et al (18) with the author's permission).

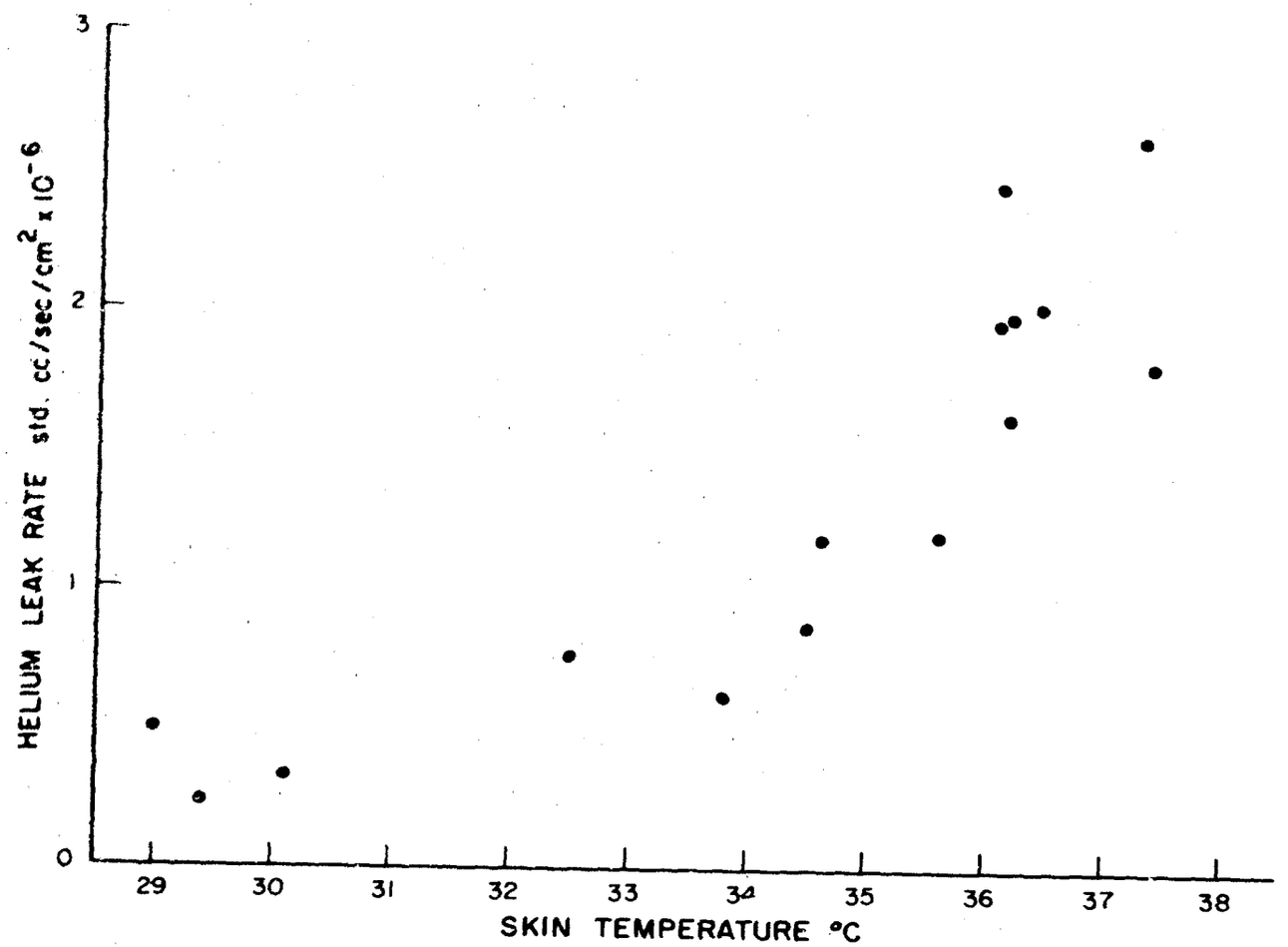

Figure 4-2 Relationships of helium leak rate and skin temperature measured in forearm skin.

Part 4

General Discussion and Conclusions

Relationship of skin blood flow to skin temperature.

The role of skin blood flow in the convective transfer of heat has been reviewed by Hertzman (64). A form of the Fick equation may be used to calculate the convection heat transfer (7):

$$H = F \times k \ (T_1 - T_2)$$

where H : heat transfer

F : blood flow

k : specific heat of blood

T_1 : temperature of the blood before heat loss

T_2 : temperature of the blood after heat loss.

Theoretical relations of blood flow to skin temperature are shown in figure 4-1 (18). Points on the curves were calculated from the following assumed values: heat transfer = 45 Kgcal/ m^2/hr/ °C/cm; effective skin thickness = 1 mm; temperature of venous blood leaving the superficial venous plexus 0.5°C higher than that of the skin surface. The curves show the required relations between the rates of blood flows and skin temperatures to maintain heat transfers and each of the respective core temperatures constant. The numbers at the top of each curve give the core temperatures.

Figure 4-2 is a plot of helium leak rates on the ordinate against skin temperature. Data from measurements on four subjects in nine experiments were used. In all cases, the helium leak rates and skin temperatures were spontaneous responses in resting subjects during exposure to increased ambient temperature. All of the points represent data sampled 10 minutes after the beginning of breathing of 80 per cent helium and 20 per cent oxygen. This sampling time was selected so that it would occur shortly after the last part of pulmonary wash-out and before the complete saturation of skin water with helium at the pHe of arterial blood, i.e. before the plateau phase of the helium leak rate curve. The distribution of points is similar to the curve for a core temperature of 37°C in figure 4-1.

The composite plot in figure 4-3 was constructed to display data from figure 5 in the paper by Senay et al (18) as solid dots giving the forearm skin temperatures against the ordinate on the left for values of "pulse amplitude" measured

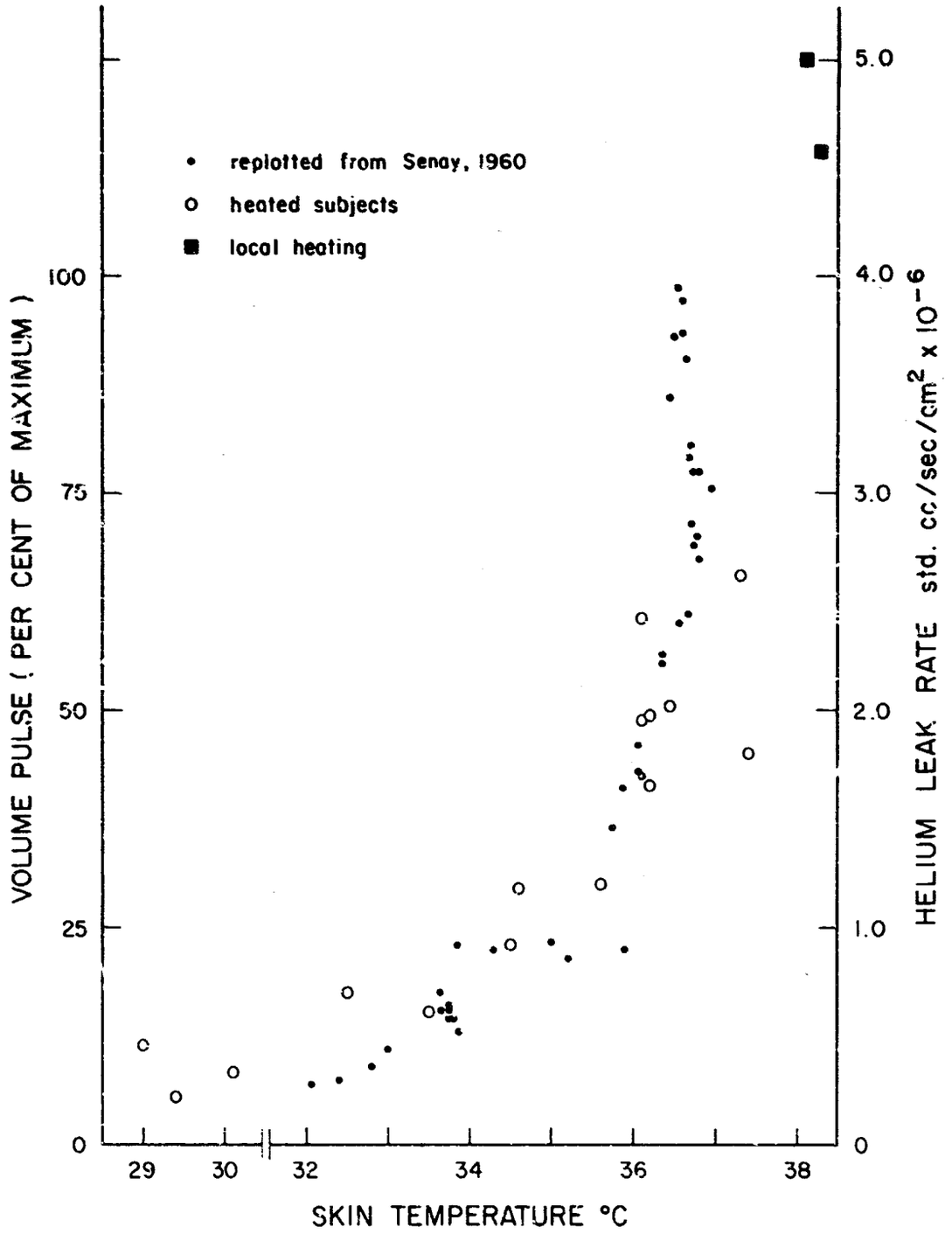

Figure 4-3 Composite plot of volume pulse data from Senay, et al (18) and helium leak rate data from figure 4-2 against the same abcissa showing skin temperature. Subjects in both experiments were at rest and exposed to rising ambient temperature.

with a photoplethysmograph. Our data from figure 4-2 are plotted as open circles on the same abcissa but against the ordinate on the right in units of helium leak rate. The two filled squares in the upper right corner of the plot are points from two of our experiments on the effects of local heating of one forearm similar to the example shown in figure 3-16. The ordinate on the right of figure 4-3 was adjusted so that the highest helium leak rates obtained in our subjects exposed to general body heating were comparable to the maximum pulse amplitudes obtained in Senay's study on subjects similarly exposed.

The congruity of pulse amplitude (Senay's index of skin blood flow) and helium leak rate, measured 10 minutes after the start of helium breathing, in their relation to skin temperature holds reasonably well in resting subjects exposed to increased ambient temperature. Both sets of data illustrate the effect of evaporative cooling of the skin when ambient temperatures rise enough to evoke sweating. Pulse amplitudes and helium leak rates continue to increase with rising ambient temperature, but the skin temperatures rise very little. Our plethysmographic measurements have confirmed the observations of others (10) that forearm blood flow rates are increased in subjects exposed to general heating. Barcroft et al (20) and Edholm et al (34) have presented convincing evidence that the increased forearm blood flow under these conditions is confined to skin.

Mechanisms of cutaneous vascular control.

Adrenergic vasomotor tone mediated by sympathetic nerves has been shown to be the dominant factor controlling resistance vessels in the skin of the hands and feet and their respective digits. Sympathetic denervation or blockade of appropriate peripheral nerves with local anesthetic produces immediate maximal vasodilatation in these regions (10). Skin of the arms, legs and trunk that is similarly denervated or blocked has an increase of blood flow, but its magnitude is only about one third to one half of the maximum blood flow that can be induced by general body heating when nerve function is intact. Thus the larger fraction of the maximum flow requires active participation of nerve traffic for its full manifestation. Details of current information on the mechanisms mediating this "active" vasodilatation are summarized in Part I of this report.

Local factors in the control of skin blood flow.

When the temperature gradient through the skin is reversed by local heating, the initial vasodilator response is in the most superficial vessels. Evidence for this view is furnished by direct counts of the number of open capillaries on cool and heated skin (13) and from data obtained on helium leak rates during local heating of one forearm in subjects exposed to comfortable ambient temperatures (see Part 3-4(e)). Dilatation

of superficial capillaries is followed within a few seconds by a large increase in forearm blood flow that is sustained for at least two hours if the level of heating is high, e.g. an arm bath temperature of 45°C (65). At bath temperatures of 38° to 42.5°C, the period of vasodilatation began to die away after 60 minutes despite continued local heating. In our experiments with local heating of one forearm with hot air, both increased forearm blood flow and increased helium leak rate persisted throughout the period of heating.

Local cooling of small skin areas of subjects at rest and sweating in a hot environment has been demonstrated to inhibit the usual vasodilatation that occurs in skin heated by the ambient temperature (18). Sweating that was measured distal to the point of application of the water-cooled thermode was approximately equal in the two forearms. This was taken by the authors to mean that thermal reflexes from skin areas in contact with the thermode were "negligible and beyond detection." This is in accord with the findings of Crockford et al (61) who demonstrated that a ring of local anesthetic proximal to the heated area of a forearm did not affect vasodilatation in the heated area nor the spread of the dilatation distally into the unheated portion. Anesthetic injected between the heated and unheated portion blocked the distal spread of the response. This observation suggests that an axon reflex, or conduction through a local nerve net, might be involved in the spread of the dilatation. However, the dilator response to local heating still occurred in patients with degenerated sympathetic and somatic nerves. Any "nerve fibers" concerned in such an information transfer must be independent of the somatic system and of the sympathetic neurons with cell stations in the paravertebral ganglia.

As an alternative explanation, Crockford et al (61) suggested that the mechanism may be one of arterial conduction, the stimulus being conducted in the smooth muscle of the subcutaneous arterial plexus. If this suggestion is taken to be an example of the type of propagation of the myogenic autoregulation referred to by Hilton (66), it is still necessary to identify an initiating process. An interesting observation made by Crockford et al may illustrate and support the hypothesis that the dilator process begins by relaxation of precapillary sphincters of the most superficial dermal capillaries.

They noted that the area treated with infra-red radiation developed erythema and increased blood flow, while in the adjoining distal area, the blood flow increased without visible redness of the skin. Thus the dilatation of superficial capillaries and increased filling of the subpapillary venous plexus occurred only in the area that was heated. In the unheated area to which the dilatation of resistance vessels

spread, the resistance to blood flow in the superficial capillaries remained high. The increased blood flow in the unheated area was thus confined chiefly to the deeper layers of the skin.

In the heated area, the opening of more superficial capillaries and the relaxation of smooth muscle in resistance vessels probably occurred at very nearly the same time but through the operation of different mechanisms. The dilatation of resistance vessels could have been mediated by one, some, or all of the following: 1. reflex inhibition of α-adrenergic sympathetic discharge; 2. reflex excitation of β-adrenergic sympathetic discharge; 3. myogenic relaxation in response to the pressure-drop produced by opening of more capillaries in the vicinity of sweat glands and the superficial layers of the skin. Precapillary sphincters are not innervated, nor are the single smooth muscle cell units in physical contact with other smooth muscle cells. Therefore the opening of additional capillaries is not mediated directly by any change of nerve activity nor by an inhibitory process spreading through arteriolar smooth muscle.

The relationship of helium leak rate to skin blood flow.

When helium leak rate is measured continuously from the beginning of helium breathing, the escape of helium from the skin surface can be detected within 30 seconds (44). Our measurements showed that young, fit subjects who were non-smokers excreted helium from the skin in detectable amounts as soon as 18 seconds after the onset of helium breathing; older subjects (59+ years) rarely were found to leak helium through the skin before 45 seconds after the start of helium breathing. In all subjects, the helium leak rate curve displayed rapid rise during the first 10 to 15 minutes and then started reaching a constant value after 15 minutes. In resting subjects at about 25°C ambient temperature, the events determining the slope of the rising rate of helium leakage are chiefly related to the rate at which pHe increases in alveolar gas. The inflection of the helium leak rate curve that occurs at about 15 minutes after the onset of helium breathing probably marks the time of near-saturation of arterial blood with helium at the pHe of alveolar gas very close to the end of the period of pulmonary wash-out. Verification of this hypothesis requires measurement of helium in arterial blood and in expired air; our analytical system was not suitable for accurate measurements on gas samples with high concentrations of helium nor measurement of helium in liquid samples.

It is likely that the height of the flat portion of the helium leak rate curve is determined by the factors that limit the rate of diffusion of helium from the blood through the tissues to the skin surface. Both Adamczyk et al (44) and Klocke

et al (53) came to the conclusion that the transfer of helium and other fixed, inert gases through the skin is a diffusion-limited process. Klocke's equation:

$$\dot{V} = K\frac{A}{h} \cdot \frac{\alpha}{\sqrt{MW}}$$

where $\dot{V}$: rate of gas transfer through skin

K : a constant

A : effective capillary surface area

h : thickness through which gas must diffuse

α : the solubility of the gas

MW : molecular weight of the gas

applied when his subjects had been breathing helium mixture long enough to be in a steady state. During any experiment, α and MW remained constant; variations in K A/h determined the amplitude of $\dot{V}$. K A/h was varied, in Klocke's experiments, by raising the temperature of the water around the plastic cylinder in which the subject's arm was immersed. The probable order of events was: heating of the skin----> increased local metabolism ----> relaxation of superficial precapillary sphincters (metabolic autoregulation)----> decreased resistance to flow----> drop in arteriolar pressure and local wall tension----> relaxation of arteriolar smooth muscle (myogenic autoregulation)----> increased skin blood flow. The factor responsible for the ensuing increased helium leak rate was the increased effective capillary diffusion surface resulting from relaxation of precapillary sphincters. The contribution of increased blood flow was merely the filling of the additional capillaries. The possible role of ipsilateral segmental vasodilator reflexes has been omitted from the above sequence on the ground that the contribution is small in forearm skin and, in fact, may not be an essential feature of the complete response as shown by studies on denervated or nerve-blocked subjects (10).

In resting subjects responding to increased ambient temperature, the local vascular response is combined with neurally mediated vasomotor changes of central origin resulting in arteriolar dilatation and activation of sweat glands. With the onset of sweating and evaporative cooling of the skin surface, fewer superficial capillaries remain open. The larger fraction of the increased skin blood flow filled the capillaries distributed to sweat glands and their ducts. This phenomenon was illustrated in our experiments with atropine blockade of sweat secretion (Part 3-7 and figure 3-13). The control arm sweated freely in the hot environment. The atropinized arm did not sweat, the skin temperature was well above that in the control

arm, and the helium leak rates differed in direct relation to the skin temperatures. The differences in blood flow reflect the considerable reduction in vascular resistance in the arm with the higher skin temperature.

The distribution of skin blood flow during the "heat-loss" response.

In subjects exposed to rising ambient temperature at low humidity, the first two stages of vasodilatation in the skin are associated with a modest rise in skin temperature but little or no increase in the number of open superficial capillaries. The major site of decreased vascular resistance in the second stage of increased skin blood flow is in the vessels that arise from the arterial plexus at the dermal-areolar junction. Dilatation of the vessels supplying the coiled portion of the sweat glands slightly precedes the onset of sweating. Evaporation of sweat keeps the skin temperature well below that of the environment. Under these conditions, the resistance to flow provided by the precapillary sphincters of the capillaries in the dermal pegs remains relatively high. The dilated vessels of the sweat glands and their ducts serve as a functional shunt that provides adequate convection heat transfer.

An increase in core temperature, ambient air temperature or radiant heat burden may increase skin temperature. At forearm skin temperatures in the range of 36.0° to 36.5°C there is a dramatic increase in helium leak rate and pulse amplitude (see figure 4-3). When local heating is used to elicit the response to rising skin temperature (see figure 3-16), the circulatory changes are not seriously complicated by general reflex vasomotor changes associated with heat-loss, and the consequences of decreased vascular resistance in the most superficial vessels can be more easily isolated. We have concluded that the factor most directly concerned with involvement of superficial vessels in heat-loss responses is a form of autoregulation and that the adequate stimulus for the response is heat. Whether the mechanism of its action is a direct effect of heat upon the smooth muscle unit or an indirect effect by way of the local increase of metabolism generated by the rise in temperature cannot be decided on the basis of evidence now available.

Conclusions.

1. The rate of leakage of helium through the skin is directly proportional to the capillary area available for diffusion and inversely proportional to the distance from the diffusing surface to the skin surface. When the number of open superficial capillaries increases, the local perfusion rate increases. Therefore the helium leak rate may be used as an index of blood flow distribution in the skin.

2. If some of the skin blood flow is shunted through channels other than the superficial capillaries, the rate of helium leakage is small relative to the total skin blood flow. The prime example of this effect was demonstrated by the small, fixed rate of helium leakage from finger skin during very large increases of blood flow in heated subjects, while simultaneous measurement of changes in blood flow and helium leak rate in the forearm of the same subject showed a roughly linear increase of helium leak rate with increasing forearm blood flow.

3. The distribution of blood flow in forearm skin is very little affected by barostatic reflexes such as those involved in the redistribution of peripheral resistance during simulated upright posture produced by lower-body negative pressure in reclining subjects.

4. Circulation of increased amounts of blood to the vessels supplying the coiled portion of sweat glands and the sweat gland ducts provides a functional blood shunt during response to general body heating. Little decrease of resistance to flow occurs in the superficial capillary bed when the skin is cooled by evaporation of sweat.

5. Interference with the evaporation of sweat in subjects responding to high ambient temperature or direct application of heat to the skin increases the number of open capillaries as indicated by increased helium leak rate, erythema, and direct counts of visible capillary loops.

6. The precapillary sphincters of the most superficial distribution in the skin do not dilate in response to any of the various changes of nerve activity involved in the heat-loss response. They are controlled by autoregulation in response to local heat or to some feature of the metabolic response to a change in heat.

Recommendations.

1. Studies should be carried out to determine the possible usefulness of measurements of helium leak rate to plastic surgeons interested in the rate of revascularization of skin grafts.

2. Studies should be carried out to determine the possible role of dilatation of superficial skin vessels in heat exhaustion and heat stroke. Both effective blood volume and lowered total peripheral resistance may be involved.

3. Current evidence suggests that successful acclimatization to heat requires an increase of blood volume. The retention of salt and water, which are the first steps in the generation of a larger blood volume, are initiated by persistent circulatory stress with decreased renal blood flow as an

essential feature. It might be useful to know whether or not acclimatization to heat occurred more rapidly and more consistently in subjects in whom the heat stress included judiciously limited increases in skin temperature.

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Appendix I

Equipment and General Procedures

1. Temperature-controlled room. An insulated room 6' x 10' provided with a refrigeration unit and electric heaters was used as the experiment chamber in which the subject was comfortably seated or reclining. Temperature control was ±1.5°C over the range 5° - 48°C when the room was used with the subject and one observer. With the subject alone in the room, the precision of temperature control was approximately ±1.0°C.

All recording equipment was outside the temperature-controlled room. The only equipment within the room, other than air lines, wrist and arm cuffs and strain gauges, was a scintillation detection probe, probe support and rate meter. All plethysmograph controls and recording equipment were outside the temperature-controlled room. Verbal directions to the subject, etc., were given by way of an intercommunication system.

2. Plethysmograph control system. The control system for air pressure supply to the wrist cuffs and collecting cuffs consisted of three air tanks, an auxiliary air pump with reducing valves, pressure gauges and solenoid valves. The system was arranged for use of external air supply or internal air pump. Both manual and automatic control was provided for excluding hand circulation by inflation of wrist cuffs at 240 mm Hg, followed by repeated applications of pre-set collecting-cuff pressure (50 - 75 mm Hg) at intervals of 10 seconds or 6 seconds. Each collection period was followed by an equal interval for exhaust of collecting pressure and unimpeded venous drainage.

3. Vacuum system for control of arm position. A low-capacity, diaphragm suction pump outside the temperature-controlled room was connected by copper pipe to a 10-gallon reservoir tank inside the room. Neoprene bags 10" x 10" and 6" x 6" partly filled with sand and connected to the vacuum system through internal, gauze-covered, perforated, polythene tubes 3" x 1/2" served as support for the elbow and hand of each arm of the seated or semi-reclining subjects. The arms were placed comfortably at the level of the sternal angle and supported by molding a large bag about the elbow while the subject grasped the smaller bag in his hand, dorsum up and slightly dorsiflexed at the wrist. With the bag adjusted to form a cupped support about the elbow in such a manner as to avoid pressure on the ulnar nerve, clamps on the tubing connecting the bags to the vacuum system were released to exhaust air from the bags. The sand bags then remained under constant suction to provide individually shaped supports for the hand and elbow with the segment of forearm elevated 1.5 to 3 cm above the plane of the supporting table.

4. Temperature measurements. Temperatures of room air, skin of each forearm and mouth were measured at regular intervals by means of thermistor probes connected through the chamber wall to a selector switch and thermistor bridge indicator (Yellow Spring Instrument Co. Model 46 TUC).

5. Construction of mercury-in-rubber strain gauges. The strain gauges used for measurement of changes in forearm blood volume during venous occlusion were modified from the description of Eagan (67) based upon the original Whitney description (21). Each gauge was connected as the variable arm of a resistance bridge as illustrated in the accompanying figure. Each gauge had a winding of 36-gauge, insulated copper wire located on the mounting of the lucite gauge bracket shown. The length of copper wire was empirically determined by finding the amount of wire which reduced to a minimum the influence of temperature change on gauge calibration. Owing to the dissimilarity of the non-linear functions of change in resistance with change in temperature for copper and for mercury, the internal correction afforded by these "temperature-compensation" copper windings was limited to a relatively narrow range.

The useful life of a mercury-in-rubber strain gauge, with its temperature compensation winding, was found to be extremely variable - especially when it was used repeatedly in temperatures higher than 28 to 34°C. Gauges used at 25°-27°C lasted about 2 months. Each strain gauge was fabricated from a length of rubber tubing having a lumen of 27 gauge and a wall thickness of 0.015". Gauges of various lengths were tried, including those mounted as doubled tubes yielding approximately 40 cm of working length covering the entire circumference of the mid-portion of the forearm, to short gauges 5 cm in length forming a short arc in the forearm circumference, and secured at the ends of the gauge mount by a varying length of flexible bead chain to complete the circle about the arm. The most satisfactory gauges were those made from 5-cm lengths of rubber tubing. Fine insulated copper wire was used to connect each gauge and its temperature-compensation winding to a 3-prong plug. The plug was fastened to the subject's upper arm to prevent disturbance of the gauge. A mating connector provided input to the resistor bridge and to the 5 PI, Low Level preamplifier of a Grass Model 5 polygraph.

6. Calibration, balancing and recording procedures. Calibration was accomplished by suspending the gauges from the upper member of a micrometer-driven clamp. The lower end of the gauge was attached to a 10-gram weight and clamped to secure it at the length produced by 10 grams of tension. The resistance bridge was then brought into balance as shown by the position of the recording pen of a Grass polygraph. The input from the resistance bridge was through the 20 K, D.C. impedance terminals of the Low Level 5 PI preamplifier

of the polygraph. When the recorder pen position indicated bridge balance (zero current flow) at all sensitivity ranges on the preamplifier, the micrometer screw on the calibration clamp was turned to produce a gauge extension of 1 millimeter in incremental steps of 0.1 millimeter each. A range of usable excursions of the recorder pen was found by adjustment of the sensitivity of the preamplifier. Subsequent adjustments of the recorder during the course of the experiment were accomplished by means of the linear preamplifier sensitivity control without disturbing bridge balance.

After calibration of the gauges on the micrometer rack, the gauges were applied to the forearm at a circumference halfway between the tip of the olecranon and the styloid process of the ulna. The gauges were secured by the required length of bead chain between the free and the adjustable portion of the lucite gauge mount. Adjustment of gauge tension was made by appropriate turning of the tangential lead-screw of the gauge mount. A tension of approximately 10 grams was produced by adjusting the screw until the recorder pen reached and remained on the same base line as that used for gauge calibration.

Appendix II

Forearm Blood Flow Gauges

1. Design, performance analysis and calibration of a light-weight, capacitance gauge for forearm plethysmography, 1966-1968.

This part of the report describes an electrical capacitance gauge for use in plethysmographic measurement of fractional blood flow. Fractional blood flow is defined as follows:

$$FBF = \frac{1}{Vol_a} \frac{dVol_a}{dt} \qquad 1 - 1$$

where Vol_a is the volume of the limb segment. The physical dimension of fractional blood flow is time $^{-1}$; a commonly used dimension is $\frac{\text{ml BLOOD}}{\text{min 100 ml ARM}}$.

The capacitance gauge has several advantages for use in plethysmography:

1. minimum disturbance of the arm, i.e. low contact pressure over a wide range of arm volume changes
2. light weight
3. small volume
4. low temperature coefficient of capacitance

Its main disadvantages are the problems associated with accurate measurement of very small capacitance changes and the difficulty of production of well defined small circumference changes for calibration purposes. The physical process of measurement of fractional blood flow consists of the following steps:

1. Arm size to capacitance: transducer
2. Capacitance to analog signal: electronic circuitry
3. Analog signal to readout: display equipment

Arm Volume - Arm Circumference Relationships: The cross section of the mid-forearm is considered to be circular when the forearm is in a pronated position (21). Since a segment of arm is considered to have a fixed length, l, any change in the volume of the segment can be measured by measuring the cross sectional area or the circumference. For the system described here, circumference is a variable more easily dealt with than the area, of course either may be derived from the other:

$$\text{Area} \propto \frac{\text{Circ}_a^2}{4\pi} \qquad 1-2$$

Circ_a: circumference of arm segment

Therefore all arm volume changes will be viewed by means of the resultant circumference changes. The computation of fractional volume changes from fractional circumference changes is based on the following relationships. Since the arm has a circular cross section, its volume is described by

$$\text{Vol}_a = \frac{\text{Circ}_a^2 l}{4\pi} \quad . \qquad 1-3$$

The relationship between fractional change in volume to fractional change in circumference is found by simple algebra and differentiation. Time, t, is entered as a factor to relate the equation to fractional blood flow (eq. 1-1).

$$\frac{d\text{Vol}_a}{d\text{Circ}_a} = \frac{\text{Circ}_a l}{2\pi} \qquad 1-4$$

$$\frac{d\text{Vol}_a}{\text{Vol}_a\, dt} = 2\,\frac{d\text{Circ}_a}{\text{Circ}_a\, dt} \qquad 1-5$$

Thus fractional volume change is twice the fractional circumference change.

Arm Circumference to Capacitance. A parallel plate capacitor is a device whose capacitance, C, is described by the equation

$$C = \frac{E_r A}{d} \qquad 1-6$$

E_r: relative dielectric constant

A : area of plate

d : distance between plates

If a small sensor plate is placed close to the surface of the forearm at a distance d, the capacitance between the sensor plate and forearm is given approximately by equation 1-6. If a band of conductor of fixed length, Circ_B, is wrapped around a segment of forearm but separated from the surface of the arm by some dielectric, the capacitance can be described approximately by the equation for the capacitance between concentric cylinders:

$$C = \frac{2\pi E_r l}{\log \frac{Circ_{Band}}{Circ_{Arm}}} \qquad 1 - 7$$

Although it was thought that these equations would be sufficient to describe accurately the arm band characteristics, it was found that there were enough non-idealities in the bands so that the equations could only be used as guides for characterization of the bands.

For arm circumferences close to the circumference of the sensing band, equation 1-7 becomes asymptotic to the equation

$$C = \frac{2\pi E_r l \, Circ_a}{Circ_B - Circ_a} \qquad 1 - 8$$

which describes the parallel plate case, if the band were "unwrapped" from the arm. From the above guiding equations, a hyperbolic relationship between arm circumference and arm band capacitance can be expected when the active electrode of the band is close to the surface of the arm.

The first capacitive transducer constructed was a strip of stainless steel 0.005 inches thick and 0.50 inches wide which was separated from the arm by polyurethane foam 0.50 inches thick. The metal band was found to be too inflexible to conform to the shape of the arm, putting the electrode at a varying distance from the surface of the arm. Therefore a strip of aluminum foil used as a backing on the foam was used as the active electrode. Under very carefully controlled conditions, this type of arm band was functional. However any movement of any object in the proximity of the arm band introduced a capacitance change unrelated to the forearm circumference. Typical causes of capacitative noise were laboratory personnel walking past the subject, the subject's chest movements, and other measuring equipment placed near the arm. Another source of difficulty was the wire connecting the arm band to the measuring equipment. This wire acted as a capacitive sensor along its entire length, and slight movements of the wire produced large amounts of capacitive noise due to two effects: capacitive coupling of the wire to the environment, and the mechanical forces of the wire on the arm band. In addition to interfering with arm volume measurements, these sources of noise precluded any attempt at accurate calibration.

The problems mentioned above indicated the need for shielding of the electrode and improvement of the method of electrical connection to the electrode. Several different

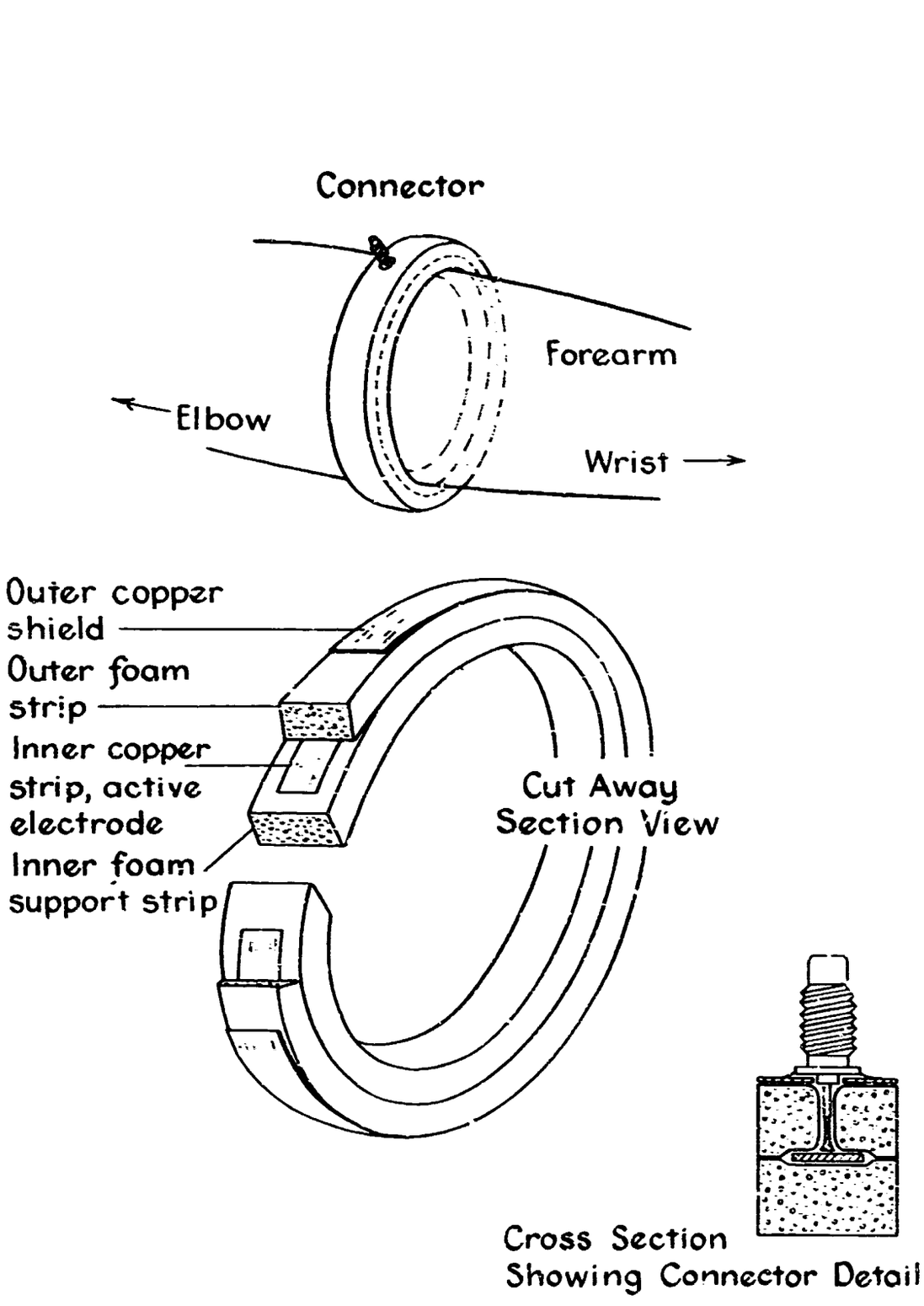


Figure II-1 Capacitance plethysmograph for the forearm.

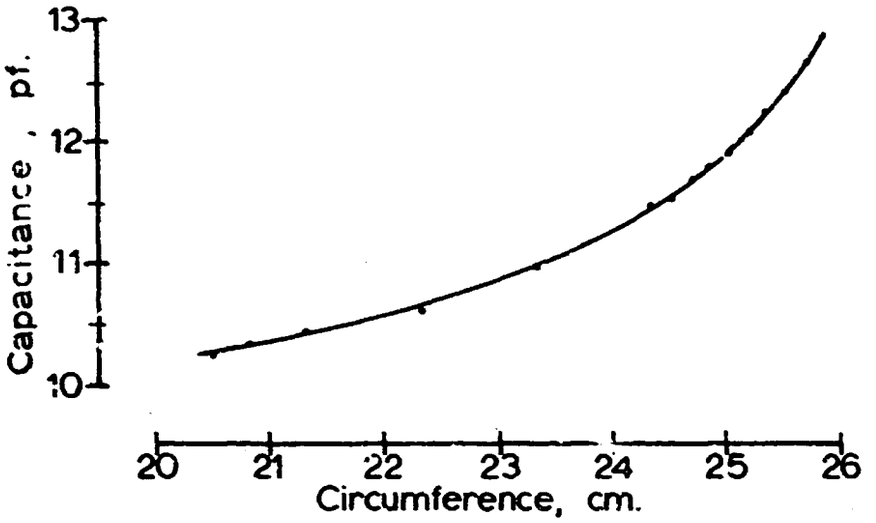


Figure II-2 Relationship of capacitance to circumference.

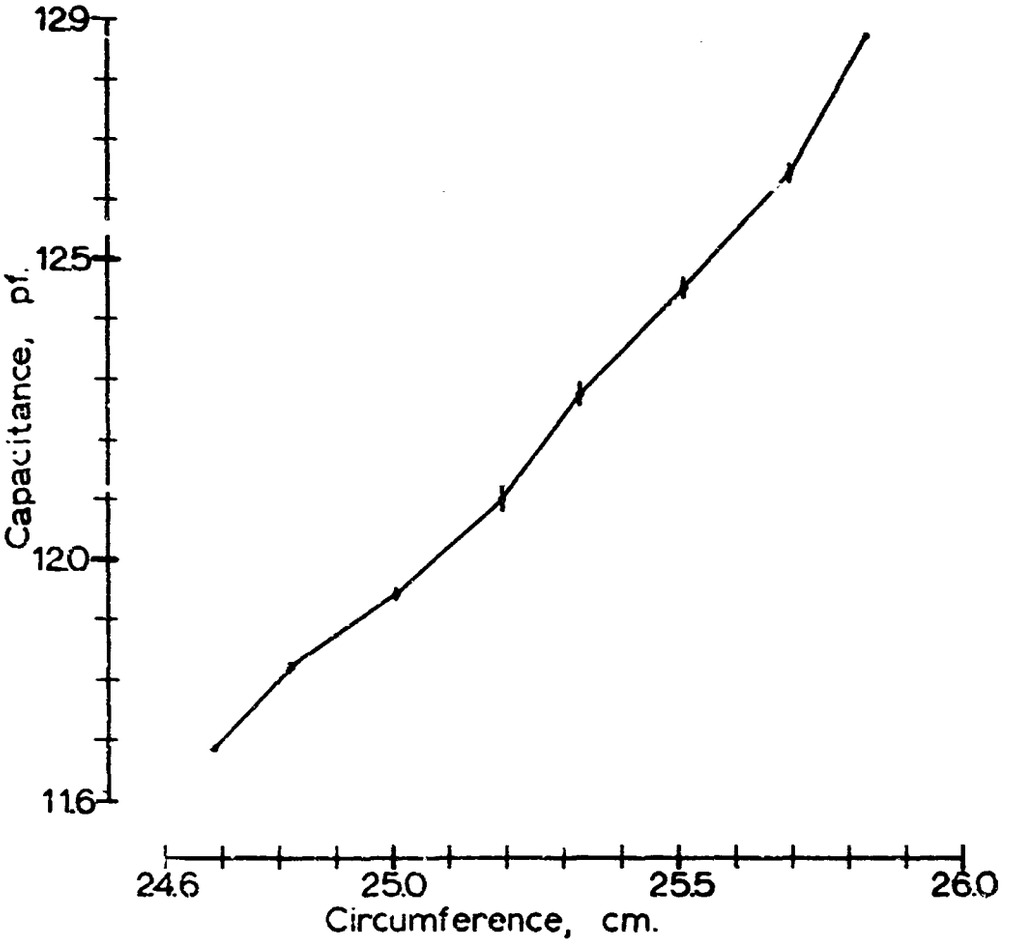


Figure II-3 Relationship of capacitance to circumference over a very small range of circumference.

approaches to the solution were attempted before the arm band illustrated in figure II-1 was designed. In this arm band the active electrode is shielded from external influences by a strip of copper tape approximately twice as wide (0.5 inches) as the active electrode (0.25 inches). A light weight coaxial connector provides a quick-connecting, noise-free terminal to the active electrode. The total gauge weight is about 5 gm. The shield is operated at ground potential (which is also the arm potential) and therefore adds about 8 to 10 picofarads between the active electrode and ground. The shield effectively prevents capacitive noise from the sources mentioned above. The arm band capacitance as a function of arm circumference is shown in figure II-2.

Relationships of circumference to capacitance of a typical arm band are shown in figure II-2. The capacitance is that which is measured at the terminal on the arm band with the gauge over the cone at the stated circumference. Over the wide range of circumferences, the relationship of capacitance to circumference is hyperbolic, as expected. Figure II-3 shows the capacitances of the arm band over a very small range of circumferences although this range is still larger than the circumference range of a typical blood flow measurement. It can be seen that in this small range the relationship between capacitance and circumference can be approximated by a straight line. From a graph like that in figure II-3, the circumference of the forearm can be derived from the capacitance of the arm band. The capacitance-vs.-circumference relationship must be ascertained for each arm band; slight differences in arm band structural dimensions produce large effects on capacitance. It should be emphasized that the information in figure II-3 may only be obtained by accurate measurement of very small changes of capacitance (about 0.01 pf) superimposed on a much larger total capacitance.

A great problem in the use of capacitive transducers is calibration so that the arm volume can be quantitatively measured. Several methods of calibration have been attempted but rejected due to excessive complexity, lack of reliability and repeatability. A system using a saline-filled bladder between the arm and the electrode was tried, but correlation of bladder volume changes with forearm volume changes was equivocal. Several types of "artifical arms" have been devised to simulate a real arm inside the arm band in a calibration procedure. The calibration method in current use employs an aluminum mandrel of circular conical section. It tapers from 31 cm to 17 cm in circumference over an axial distance of 28 cm. By placing the arm band over different circumference marks on the cone and measuring the capacitance between the cone and the arm band, one can determine the relationship of capacitance to circumference for that parti-

cular arm band.

Capacitance to Analog Signal. Four commercially available electronic devices have been used for conversion of the arm band capacitance changes to an analog signal (current, voltage, or frequency). Each will be identified below, with notes on its usefulness in this particular application.

The Decker model 902 Delta unit is a device utilizing a gas-filled tube which, when driven by a fixed-frequency oscillator, produces a DC voltage that is a function of two capacitances connected to the gas tube sensor probe. The Delta unit has rather heavy sensor probes which must have a variable capacitor mounted on them. The conversion factor between voltage output and capacitance varies as a function of the measured capacitance and the balancing capacitance. This variable conversion factor makes the calibration procedure much more difficult. The connectors on the probe are not standard coaxial connectors and are unshielded; this makes the sensor susceptible to capacitive noise. Leads from the sensing capacitor to the probe and from the oscillator to the probe have length restrictions which place constraints on instrument location. The oscillator and vacuum tube voltmeter section are large heat sources, and in temperature-controlled rooms presented undesirable added problems of temperature control.

The Lion Research Corporation C-Line instrument is a semiconductor circuit using a fixed-frequency oscillator, with a diode, resistor and capacitor network to convert capacitance changes to current or voltage changes. The C-Line has a less bulky sensor probe than the Delta unit and does not generate as much heat, but it does have non-standard connectors and requires a balancing capacitor which changes the value of the conversion factor between capacitance and voltage output. The C-Line has been used with the arm bands for plethysmographic measurements and has yielded usable comparative data. The ground connections to the C-Line form an important and sensitive part of its detecting circuit - so sensitive that touching any ground connector or any movement near a ground wire can produce an output from the C-Line many times greater than that derived from an arm volume change. This problem with the ground circuit has virtually precluded further use of the C-Line: early attempts at arm band calibration proved entirely unsuccessful.

The Kenelco Capacitance Plethysmograph uses a circuit in which the measured capacitance changes the frequency of an LC oscillator. This frequency is changed to a DC voltage by a standard FM discriminator circuit. The Kenelco does not require a special sensor probe or a balancing capacitor.

Cable length is not critical and there is good offset capability to balance out cable capacitance. The conversion factor between capacitance and output voltage does not vary within the useful range for arm band capacitance measurements. This instrument is being tested for general plethysmographic work, with arm bands as capacitance electrodes instead of the bulky double-screen cuffs supplied by the manufacturer.

The Tektronix type 130 LC meter is another instrument in which the measured capacitance changes the frequency of an LC oscillator. The type 130 LC meter is a very stable device with moderate capacitance offset capabilities. It does not require special sensors or balancing capacitors, and there is a direct readout on a meter on the front panel. The stability and simplicity of the Tektronix has made it a very useful instrument for arm band capacitance measurements especially during calibration procedures.

All of the above instruments were studied and calibrated with a General Radio (GR) precision variable capacitor type 722-ME. The measurements for figures II-2 and II-3 were taken with the Tektronix LC meter and the GR precision variable capacitor using the null method. The GR capacitor was put in parallel with the arm gauge capacitance. As the position of the arm gauge on the calibrating cone was changed, the capacitance of the GR capacitor was changed to maintain the indicator on the LC meter at its original position. The value of the capacitance change was then read from the micrometer index of the GR capacitor. Thus, the arm band was calibrated by a null method independent of the sensitivity of the instrument used.

2. Elastic force gauges: resistance/volume relationships. Control circuits and calibration.

Two important characteristics of the Whitney gauges have limited their usefulness in our studies of total forearm blood flow in subjects tested during large increases of environmental temperature. The extremely low source impedance of the mercury gauges, often less than 0.5 ohm, created difficulties of impedance-matching to the Grass and Sanborn recording equipment available to us. The fairly large negative temperature coefficient of the mercury-in-rubber gauges could not be compensated over ranges of more than 5°C by means of the copper wire compensation coil suggested by Whitney (21). Hence, during experiments upon subjects in a room being heated from 25° to 45°C over a period of 30 minutes, repeated adjustment of gauge tension and re-calibration were required for accurate measurements of forearm blood flow.

The high-impedance elastic force gauges described by Waggoner (27) are satisf ctory for direct impedance matching to our recording equipment, but simple DC circuits of the Wheatstone bridge type proved to induce resistance changes in the gauges that were not related in a simple way to gauge length. Current flow thro gh the gauges amounting to only a few µa was sufficient to produce disturbing electrolysis, migration of the electrolyte ions within the electrode paste and vigorous corrosion of the copper wires used for the electrodes.

LIST OF SYMBOLS

Vol_a	Volume. arm segment
t	time
R_g	Resistance. gauge
T	Temperature
$\rho(T)$	Resistivity. (T) indicates temperature dependence
$Circ_a$	Circumference of arm segment
Vol_g	Volume of fluid in gauge
l_a	length of arm segment

The ultimate quantity to be measured is fractional blood flow (FBF).

$$FBF = \frac{1}{Vol_a} \frac{dVol_a}{dt} \qquad 2 - 1$$

Although the electrical resistance of the gauge varies with the square of the arm circumference,

$$R_g = \frac{\rho_{(T)} \, Circ_a^{\,2}}{Vol_g} \qquad 2 - 2$$

it varies linearly with the volume of the arm segment.

$$R_g = \frac{4\pi \, \rho_{(T)} \, Vol_a}{l_a} \qquad 2 - 3$$

But the conversion factor between volume and resistance varies with temperature (eq. 2-4), which introduces temperature-dependent calibration problems.

$$\frac{dR}{dVol_a} = \frac{4\pi \, \rho_{(T)}}{l_a} \qquad 2 - 4$$

The following method was derived to obtain a system for measuring FBF independent of fluid resistivity and hence independent of temperature.

It can be seen by combining equation 2-3 & 2-4 that the fractional volume change is equal to the fractional resistance change, and that the relationship is <u>independent of temperature</u>.

$$\frac{dVol_a}{Vol_a} = \frac{dR_g}{R_g} \qquad 2 - 5$$

Note that this is equivalent to

$$d \log Vol_a = d \log R_g \qquad 2 - 6$$

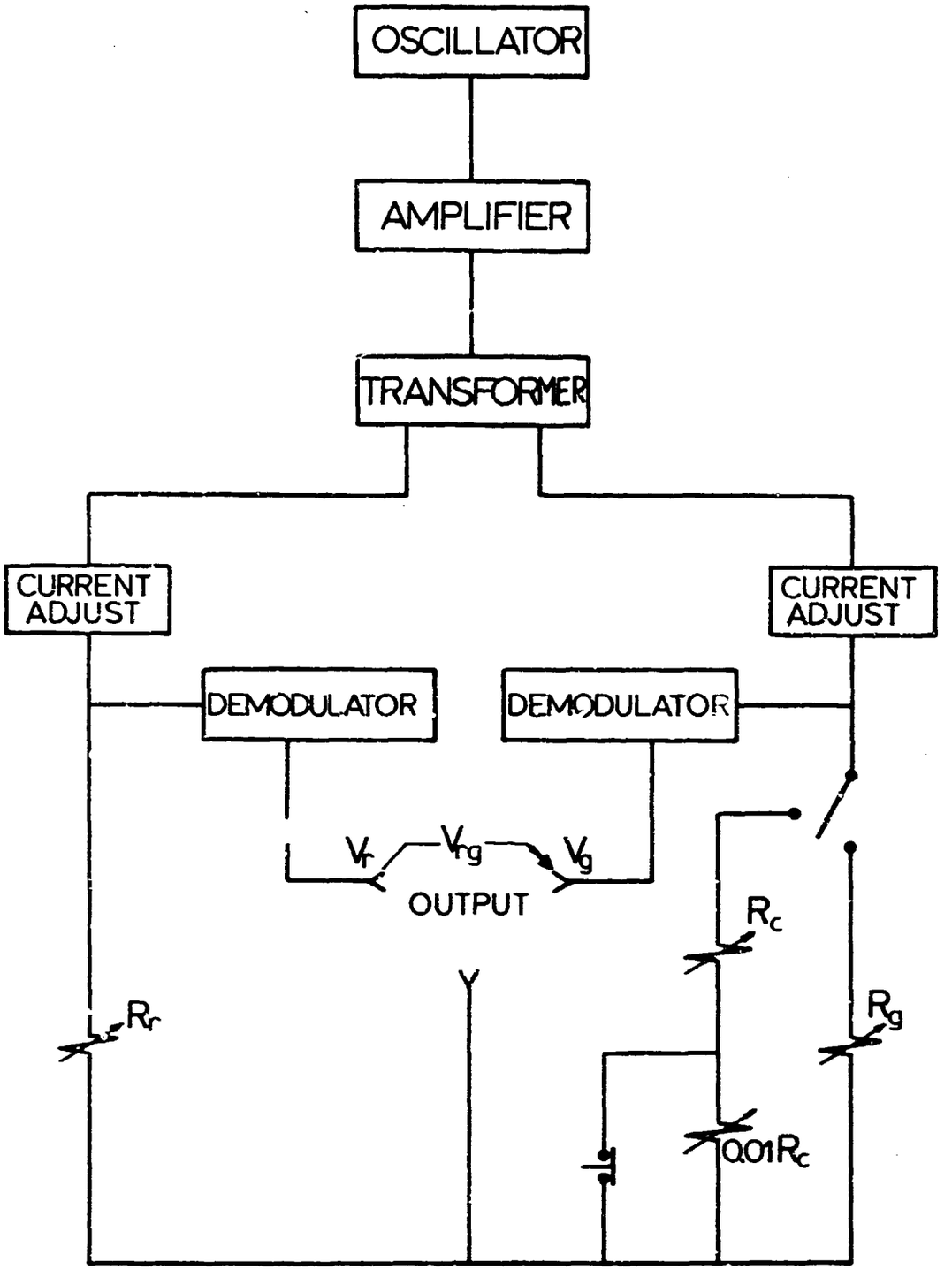


Figure II-4 Block diagram of control circuit for high impedance resistance gauges.

Therefore if the fractional resistance change can be measured, the fractional blood flow can be obtained from it directly.

$$FBF = \frac{1}{Vol_a}\frac{dVol_a}{dt} = \frac{1}{R_g}\frac{dR_g}{dt} \qquad 2 - 7$$

A circuit, shown in block diagram, figure II-4, was therefore designed and constructed for use in measuring fractional resistance changes, avoiding the DC polarization problems previously mentioned. The circuit produces a DC voltage which is proportional to the gauge AC impedance. The basic operation of the circuit is to pass an AC current, I_g, through the gauge and measure the voltage, V_{ac}, produced across the gauge.

$$V_{ac} = I_g R_g \qquad 2 - 8$$

The forced current comes from a high impedance AC current source which assures its constant value. A frequency of about 1 kHz has been found acceptable for this use. The demodulator section of the circuit produces a DC output voltage proportional to the magnitude of the AC voltage appearing across the gauge.

Changes of gauge resistance are measured by comparing the gauge output voltage with a fixed reference voltage produced across a fixed resistor (see fig. II-4). V_r and V_g indicate the voltage difference V_{rg}. This voltage is used for both calibration and blood flow measurements. The magnitude of I_g is adjusted so that the gauge output voltage is equal to the reference output voltage (in this case 1.0 volt). This is usually done by setting V_{rg} equal to zero. This should be done by varying only. V_{rg} is checked and reset equal to zero just prior any series of blood flow measurements.

As a check on calibration, a precision variable resistor, R_c, with readout on a 3-digit dial is substituted for the gauge resistance. With I_g remaining fixed at the value previously determined to be appropriate for the gauge resistance, R_c is adjusted so that the "gauge" output voltage is equal to the reference output voltage. With R_c adjusted properly, there will be no change in output voltage when R_c is substituted for R_g. In this condition the impedance of the gauge is equal to R_c. Another resistor of value 0.01 R_c put in series with R_c provides a well-defined calibration signal. Since the fractional resistance change is 0.01, the signal is that equivalent to a fractional volume change

of 0.01. From the value of the fixed reference voltage and the voltage difference from reference output to gauge output the fractional voltage change is known, hence the fractional volume change.

$$FBF = \frac{dVol_a}{Vol_a dt} = \frac{dV_g}{V_g dt} \qquad 2 - 9$$

3. An improved technique and circuit for mercury-in-rubber strain gauge plethysmography.

In Appendix II, 2 and in (26), it was shown from physical principles that gauge fractional resistance changes are equal to forearm fractional volume changes independent of temperature. Although this relationship was derived for a high impedance gauge, it also holds true for a low impedance mercury-in-silastic gauge. However, for this relationship to be utilized in practice, two conditions must be observed:

1. The gauge must go around the arm exactly 1.0 times.
2. The device that measures gauge impedance must not be significantly affected by series or shunt impedances in the cables or measuring circuits.

Gauge construction and mounting:

The gauges are constructed from Dow-Corning Silastic medical-grade tubing of 0.025" internal diameter and 0.047" external diameter. Silver solder wire is used for the plug in the end of the gauge. This combination of Silastic tubing and silver solder wire results in an increased life of the gauge compared with rubber tubing and copper wire gauges. These gauges have a nominal resistance of 0.75 ohms for a 25-cm gauge.

In order to meet the first condition stated above, the silver solder at the ends of the gauges is bent as shown in figure II-5b. A small lucite bridge (figure II-5a) holds the gauge on the arm in such a manner that the ends of the silver solder wire which are inside the tubing are juxtaposed. Thus the small bridge holds the gauge so that it goes around the arm exactly once; it also serves as a terminal for the cable from the measuring circuit.

Resistance measurement circuitry:

Since the resistance of the copper wire from the measuring circuit to the arm gauge is significant compared to the gauge resistance, a method was devised to satisfy the second condition stated above. A four-wire system is used: two of the wires are used to force current through the gauge, and two are used to measure the voltage drop produced across it (figure II-6). This system will accurately measure the gauge resistance if the current source is stable and unaffected by gauge resistance changes and if the voltage measuring circuit draws little or no current. Under these conditions the resistance of the copper wires has no effect on the measurement of gauge resistance.

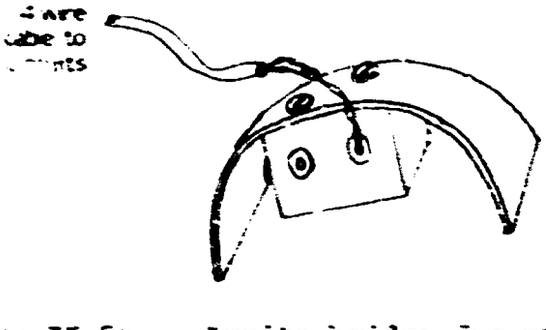


Figure II-5a Lucite bridge for gauge.

Figure II-5b Configuration of silver-solder connecting points.

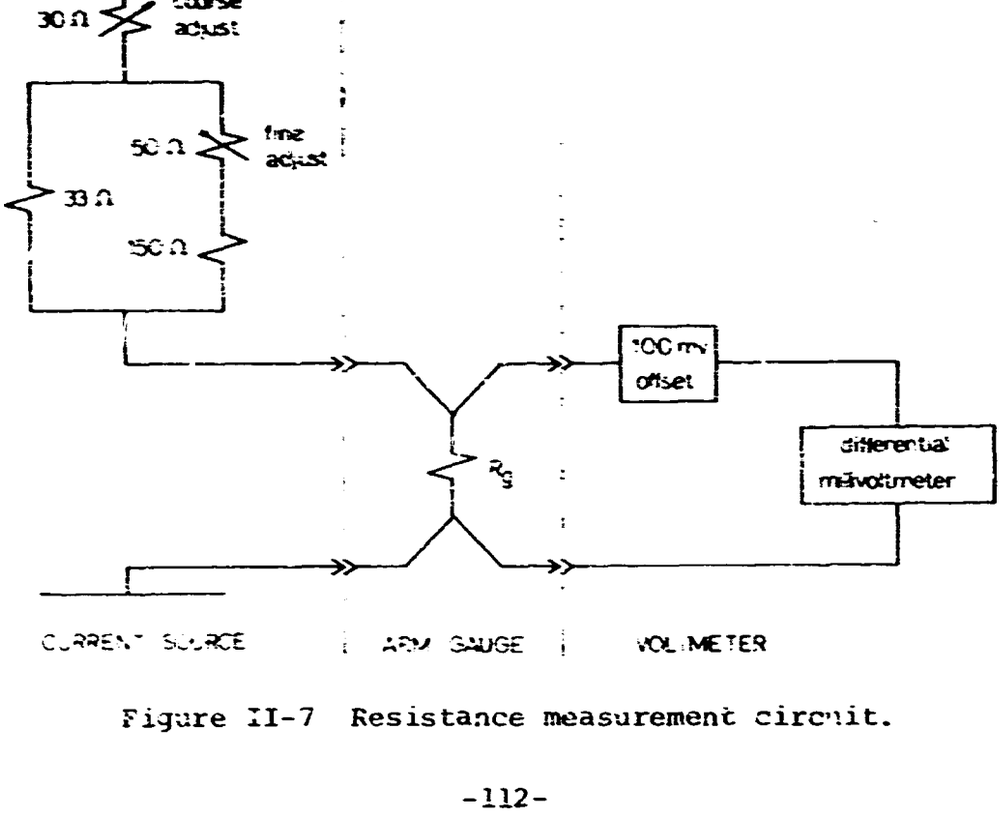
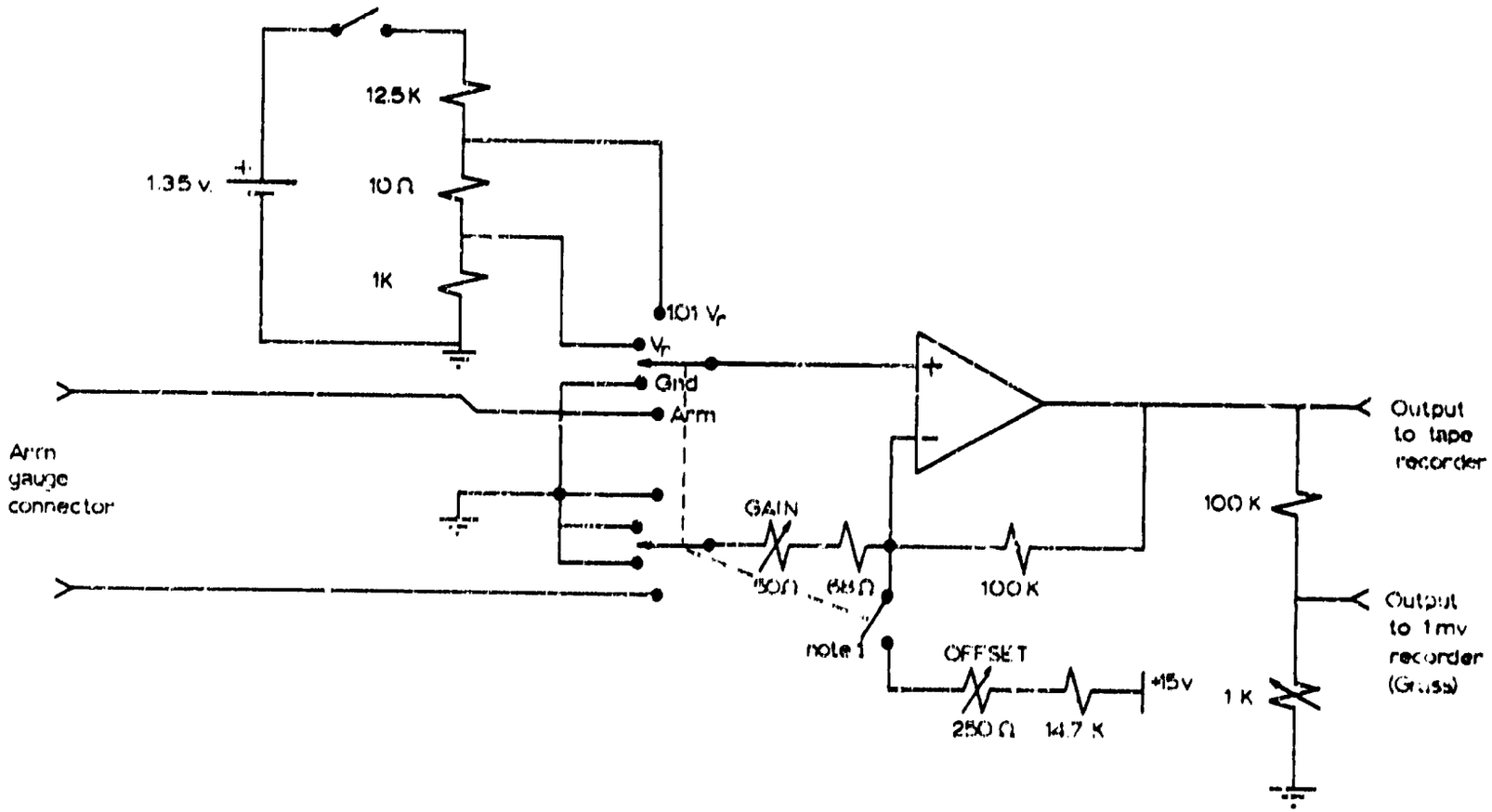


Figure II-7 Resistance measurement circuit.



note 1 switch open only in Gnd position

Figure II-8 Signal conditioning circuit.

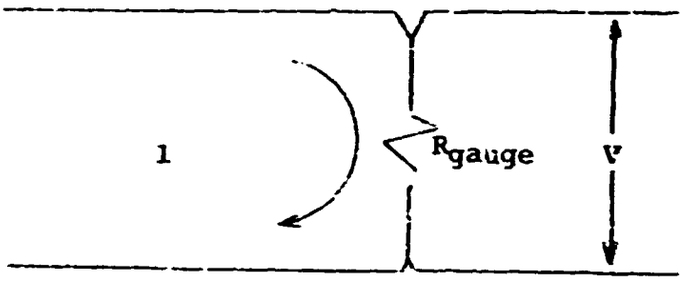


Figure II-6

The current source shown in figure II-7 can supply any gauge of length 20 cm to 30 cm with the proper amount of current to produce a 100 mv drop across the gauge. The circuit is designed to be operated from a 5-to 7-volt power supply. A conventional lead-acid storage battery may be used as a power supply.

In order to measure forearm volume change the voltage measuring circuit must provide a 100-mv offset. The Grass 5PIH preamplifier in the Grass model 5 polygraph contains all the circuitry to the right of the dotted line in figure II-7 (including the 100-mv offset). The current source and the Grass polygraph can thus provide a suitable ink-chart readout of forearm volume change. However, to record the signal on a magnetic tape recorder, additional amplification must be provided.

Signal conditioning circuit:

An operational amplifier circuit that provides voltage offset and adequate amplification is shown in figure II-8. This circuit provides suitable simultaneous output for the magnetic tape recorder and any 1-mv chart recorder (Grass).

A Philbrick/Nexus model P55AU operational amplifier is used. The recommendations of the manufacturer were followed for voltage trim adjustment and power supply characteristics. The gain of this circuit is 1000; therefore, a 1% change in arm volume will produce a 1-volt output. A four-position switch alters the circuit function to aid in adjustment of voltage trim, offset voltage, and gain. Reference voltage for offset and gain adjustments is provided by a mercury cell and resistive divider network.

Adjustment and operation of signal conditioning circuit:

The operational amplifier signal conditioning circuit is basically a millivoltmeter with offset; thus a reference voltage source may be used to adjust the circuit for proper operation. The three basic adjustments of the circuit are

made as follows: with the function switch in position 'F' adjust 'R' so that the output is 'U'.

step	F	R	U
1	Gnd	trim	null (zero)
2	V_{ref}	offset	null
3	1.01 V_{ref}	gain	1.0 volt

Steps 2 and 3 may have to be repeated in order to null the circuit in the V_{ref} position.

'Gnd' position grounds the input to the operational amplifier to aid in trim adjustment. This position is also used as a 'resting' position to protect the operational amplifier while the gauge is being changed or if any unexpected trouble occurs. The V_{ref} position applies 100 mv to the input of the circuit so that the offset may be adjusted to subtract 100 mv. The 1.01 V_{ref} position applies 101 mv to the circuit input. This is a 1% change of voltage and represents a 1% change of arm volume; thus the gain of the circuit can be adjusted to produce 1 volt output for a 1% change in input. After the equipment has warmed up, it should not be necessary to change these adjustments.

For normal plethysmographic measurements the function switch is put in 'Arm' position, and the gauge current is adjusted to null the amplifier (center the trace on a chart recorder). With the current control left in that position, the change of output of the amplifier represents the change of the arm volume from its initial value. If the trace goes off scale it should be restored using the 'fine' current control. All of the circuits are duplicated to provide for measurement of the contralateral arm; however, they utilize common power supplies and voltage reference.

Appendix III

Procedures and Equipment for Measuring Helium Flux Through the Skin.

1. Helium analysis by gas chromatograph. 1966-67.

a. The Helium Supply Systems. On the basis of Behnke and Willmon's (29) undocumented statement that "diffusion of helium outward through the skin when a helium-oxygen mixture is inhaled is of the same order as inward diffusion of helium", we avoided many cumbersome equipment problems by having our subjects breathe a mixture of 80 per cent helium - 20 per cent oxygen while we collect small helium samples from plastic chambers cemented to the skin surface. Medical grade helium-oxygen mixtures were used from standard type G cylinders supplied through humidifiers to 100 liter heavy vinyl plastic bags from which the subjects breathed. Two forms of breathing systems have been used: a closed system provided with a pump for circulating the expired gas through a soda-lime carbon dioxide absorber and back to the plastic bag, and a two-bag system which made use of the second bag via one-way J-valves to collect the expired gases prior to venting them outside the chamber in which the subjects were tested. The closed system was monitored continuously for pO_2 with a Beckman macro-oxygen electrode and Model 160 Medical Gas Analyzer. The recorder output of the Model 160 was used to trigger a solenoid valve connected to a tank of oxygen. The circuit was designed to maintain an oxygen partial pressure between 130 and 160 mm Hg in the closed system. Although the closed system was much less expensive to operate, the helium partial pressure varied between 427 and 640 mm Hg during the cycles of oxygen depletion and addition.

b. Analytical Requirements for Helium. The design of our experiments required that simultaneous measurement of total forearm blood flow, sweat rate and helium flux rate be made on both resting and exercising subjects during large changes in environmental temperature. Venous occlusion plethysmography for total forearm blood flow required the use of volume-sensing devices that would not interfere with collection of gas and sweat samples over contiguous areas of forearm skin. Elastic strain gauges are discussed in Part 2 of this report, with details of construction in Appendix II. None of the volume-sensing gauges occupied more than a 1/2" segment of forearm. Collection of helium and sweat from skin areas immediately adjacent to the gauges was accomplished by intermittent and continuous sampling respectively from plastic chambers cemented to the skin with ACMI No. 738 Ileostomy Skin Adhesive. Approximately 20 cm^2 of skin are enclosed in each chamber. Estimates

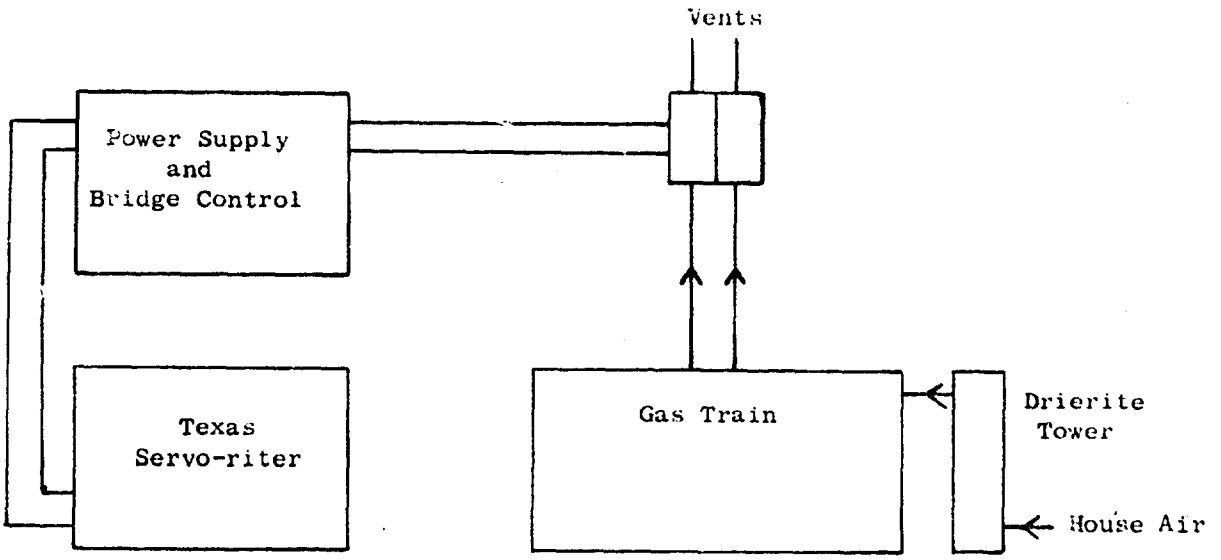


Figure III-1 Helium Analysis system.

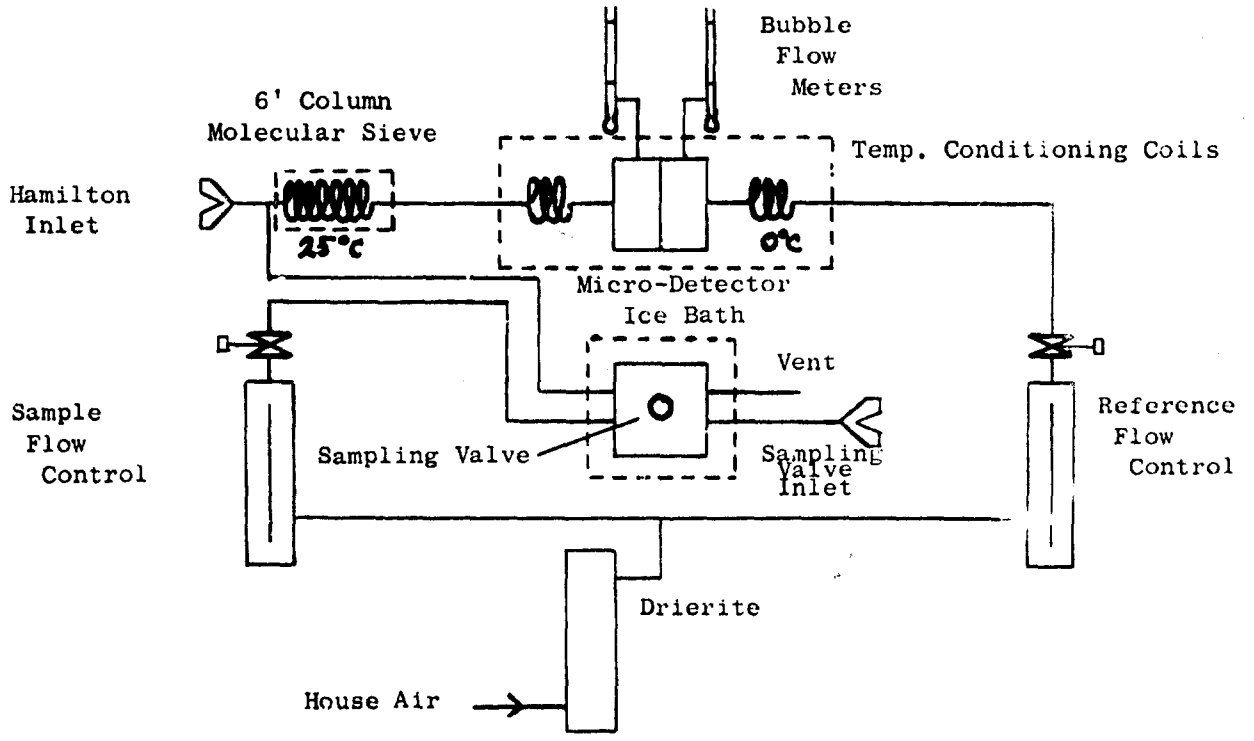


Figure III-2 Detail of gas train.

of the probable rates of helium flux in resting subjects at 20 to 25°C ambient temperature indicated that the measuring equipment would have to be capable of resolving absolute amounts of helium of the order of 0.25 μL.

A simple gas chromatograph was assembled using a 6' column of 1/4" copper tubing filled with 5A molecular sieve, 50-60 mesh in a 2 liter vacuum jar at room temperature and a GOW-MAC Model JDC 470 Microcell thermistor detector and Model 9999:D;1 power supply and bridge control unit. The detector was housed in a 2 liter vacuum jar which was filled with crushed ice at least 30 minutes before helium analyses. The output of the bridge control unit was recorded on a Texas Servo-riter with integrator.

With nitrogen as the carrier gas and also the gas used to flush and fill the gas-sampling chamber, satisfactory analyses were performed on helium-nitrogen mixtures ranging from 0.5 ± 0.1 to 10 ± 0.05 μL of helium per ml. When 1 ml gas samples were taken from gas collection chambers cemented to the skin of the forearm of subjects breathing 80 per cent helium - 20 per cent oxygen, helium was detectable in the chromatograms by a peak appearing at the usual helium retention time. However, two additional peaks of small magnitude caused slurring of both the leading and the trailing edges of the helium trace. These additional peaks were subsequently found to be those of oxygen and carbon dioxide. Separation of these contaminants could have been accomplished by using a longer molecular sieve column at the expense of almost doubling the time required for each analysis. Since we wanted to keep the analysis time below 10 minutes in order to have analyses keep pace with gas collection periods, we changed the carrier gas and gas phase of the collecting capsules to air. With air as the carrier gas, the only substance contributing a signal from the detector was helium; the amounts of oxygen, nitrogen or carbon dioxide that diffuse from the skin into the collection chamber are not sufficient to be detected as thermal conductivity differences against air as the reference gas. The system shown in figures III-1 and III-2 was further modified to reduce noise and zero-drift by replacing the GOW-MAC micro-cell, power supply and bridge control with a battery-powered Carle Model 1110 Micro Detector Control Unit and Model 1100 Micro Detector. The system included a Hamilton Model 86800 On-Column Inlet for samples of 1 to 50 μL and a Carle No. 2014 Sample Valve with two 1.0-ml loops. The sampling valve permitted precise introduction of 1.0 ml of gas at ambient pressure by charging the sampling loops with any volume of gas greater than 1.5 ml. Except during change of Drierite, the air flow was left on for continuous purge of the gas train.

The analytical procedure involved the usual equalization

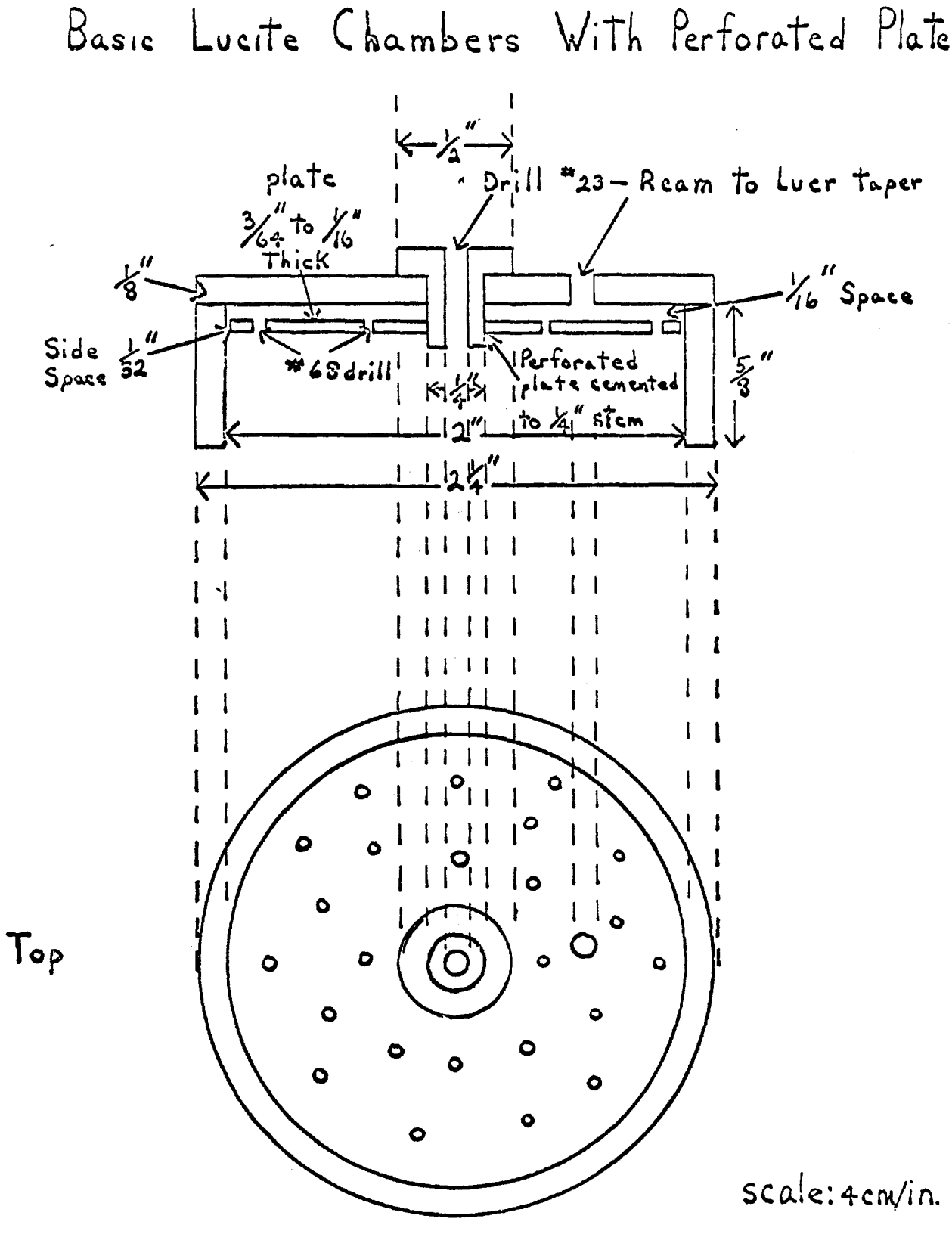


Figure III-3 Basic lucite chambers with perforated plates.

of air flows through the reference and sample sides of the detector to 8.0 ± .2 ml/min. After thermal equilibrium had been reached, final adjustments of air flow were made with calibrated bubble flow meters. Electrical balancing of the measuring bridge at a current flow of 15 m.a. was accomplished and several 5 µL samples of pure helium were delivered into the Hamilton inlet with a gas-tight 25 µL Hamilton syringe, pre-set to the fixed volume by means of a Chaney Adaptor. Since precise measurement of volume of gas samples, at the time they were withdrawn from the plastic gas collection chambers was not necessary, we used ordinary 5-cc Tomac plastic disposable syringes fitted with G 26, 1" needles. Gas samples were withdrawn at 10-minute intervals from the gas collecting chambers by inserting the needle through the serological cap of the central stopcock on the chamber. With the needle inserted to its hub, the tip was within the chamber about 2 mm above skin surface. The dead space of the syringe was flushed and the gas within the chamber was thoroughly mixed by withdrawing the syringe plunger three times to the 2 cc mark and reinjecting the gas back into the chamber. The sampling consisted of making a final withdrawal of the plunger to the 2.0 cc mark, removing the needle from the serological stopper and immediately sealing the syringe and needle by pushing the needle into a small soft rubber stopper. Both stopcocks of the plastic chamber were then opened to the atmosphere and the chamber was flushed for 30 seconds at 1 liter per minute from the house air supply. The stopcocks were closed and the next collection period was marked from the moment of closure. The gas sample in the syringe was reduced to about 1.5 cc by adjusting the syringe plunger, the rubber seal was removed from the needle and the sample was delivered into the gas sampling valve. Since this valve was vented to the atmosphere, the gas in the 1.0 ml sample loop came to ambient pressure; thermal transients were avoided by housing the entire sampling valve and sample loops in 1.5 inches of polystyrene foam covered with aluminum foil. Five seconds after one sample loop was charged, the sample was delivered into the molecular sieve column by a quarter turn of the sampling valve stem. This maneuver communicated the second, matched sampling loop with the injection port, ready for the next injection.

c. Design and Fabrication of Plastic Chambers. Figure III-3 shows a plan and elevation of the basic plastic chamber which was fabricated from clear acrylic tubing, 2" I.D. x 2-1/4" O.D. A circular plate of the same material was cemented to cover one end of the 5/8" cylinder. A central stem, machined from 1/2" acrylic rod was cemented to the upper plate and served as the means of suspending the perforated plate at a distance of 1/16" below the inner surface. A central hole and one centered 7/16" to one side were reamed to luer taper for direct connection of syringes, needle adapters or 3-way stopcocks.

Modifications of Basic Chambers for Gas and Sweat Sampling

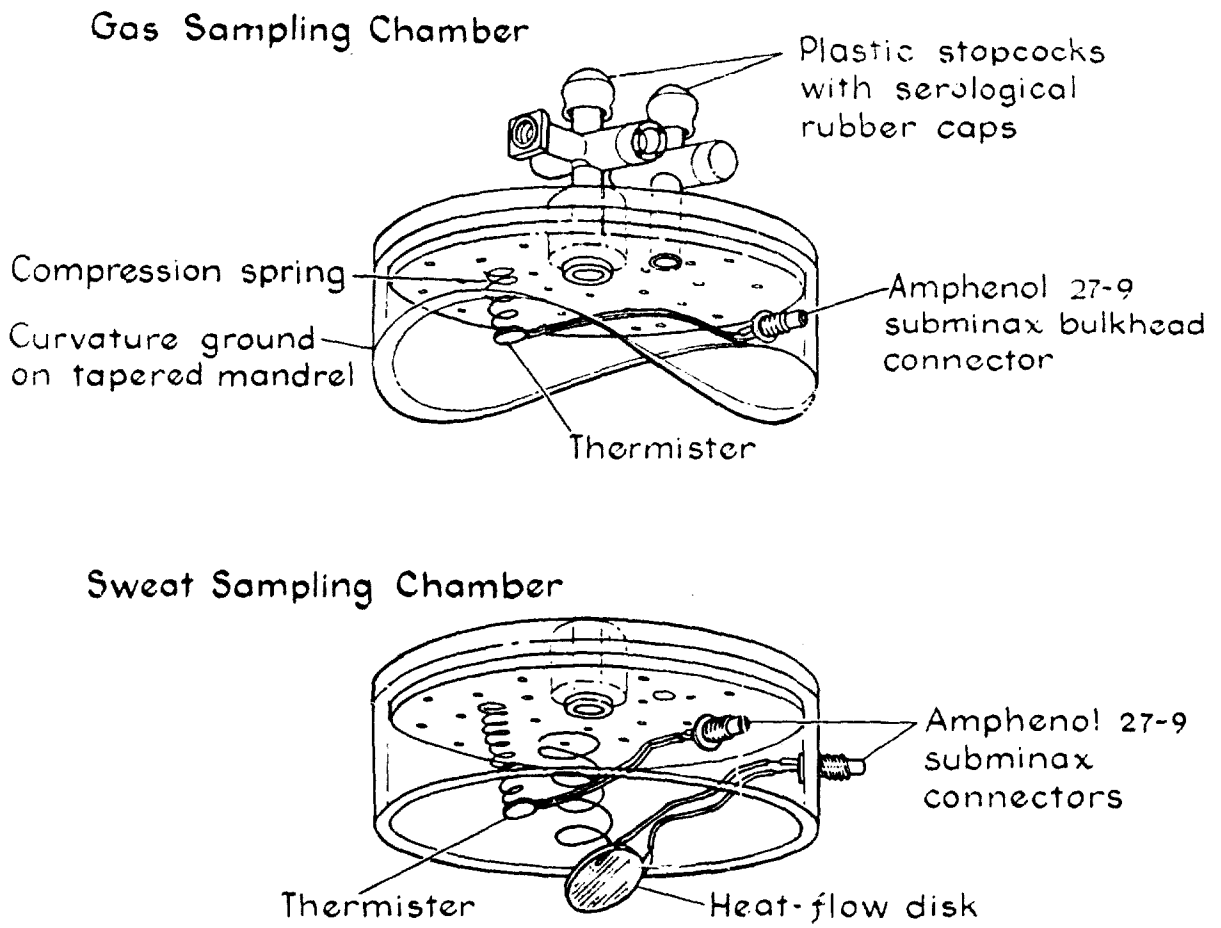


Figure III-4 Modifications of basic chambers for gas and sweat sampling.

The basic chambers were modified for collecting helium from the surface of forearm skin as shown in figure III-4a. The lower, free edges of the chambers were ground to produce a curved face that was easier to secure to the skin with surgical adhesive and the additional advantage that a slightly larger area of skin was enclosed and the volume was reduced to slightly less than half of that of the unmodified chambers. An 11-inch maple mandrel provided with a steel shaft along its central axis and supported in ball bearing pillow blocks was turned to a 5° - 32' taper with maximum diameter of 3.9". With No. 320 emery paper cemented to the surface of the mandrel and power supplied by a 1/4 H.P. motor through a Zero-max speed regulator, rapid preparation of chambers with uniform bottom curvature was accomplished.

The lead of a YSI Small Surface Temperature Probe, 400 series, No. 427 was cut to 2.5 cm and the wires soldered to the shield and central conductor of an Amphenol No. 27-9, Subminax bulkhead connector sealed into the wall of the chamber with epoxy cement. A light phosphor bronze extension spring cemented to the back of the thermistor element and to the upper plate of the chamber was added to keep the thermistor in contact with the skin at relatively constant pressure. The two openings in the top of the chamber, which had been ground to luer taper, were fitted with disposable plastic 3-way stopcocks having the vertical female connections of each plugged with miniature serological stoppers.

Effective sampling areas were estimated from weights of millimeter tracing paper cut out at the mark of the inner edge left when the chamber was pressed on the paper over a forearm or over the tapered mandrel covered with 1/8" polyurethane foam. The areas thus obtained were corrected by subtracting the area of the thermistor. Effective chamber volumes were estimated by helium dilution and gravimetrically before and after filling mounted chambers with water treated with Zepheran as a wetting agent. A systematic difference of approximately 2.1 ml, with the volumes calculated from helium dilution being uniformly lower, was not resolved. We have used the volumes obtained by gravimetric means in all calculations on the ground that failure of complete mixing of helium in the lumens of the plastic stopcocks is an unavoidable source of error in the helium dilution method.

Modification of the chambers for measurement of sweat rates involved the addition of two Amphenol Sub-minax bulkhead connectors. One of these was used for a thermistor similar to that used in the gas collection chambers; the other was connected to the shortened leads from a National Instrument Laboratories Model HF 1C, Spring-loaded heat-flow disk. Two pairs of No. 0 x 80, round-head machine screws were let into opposite

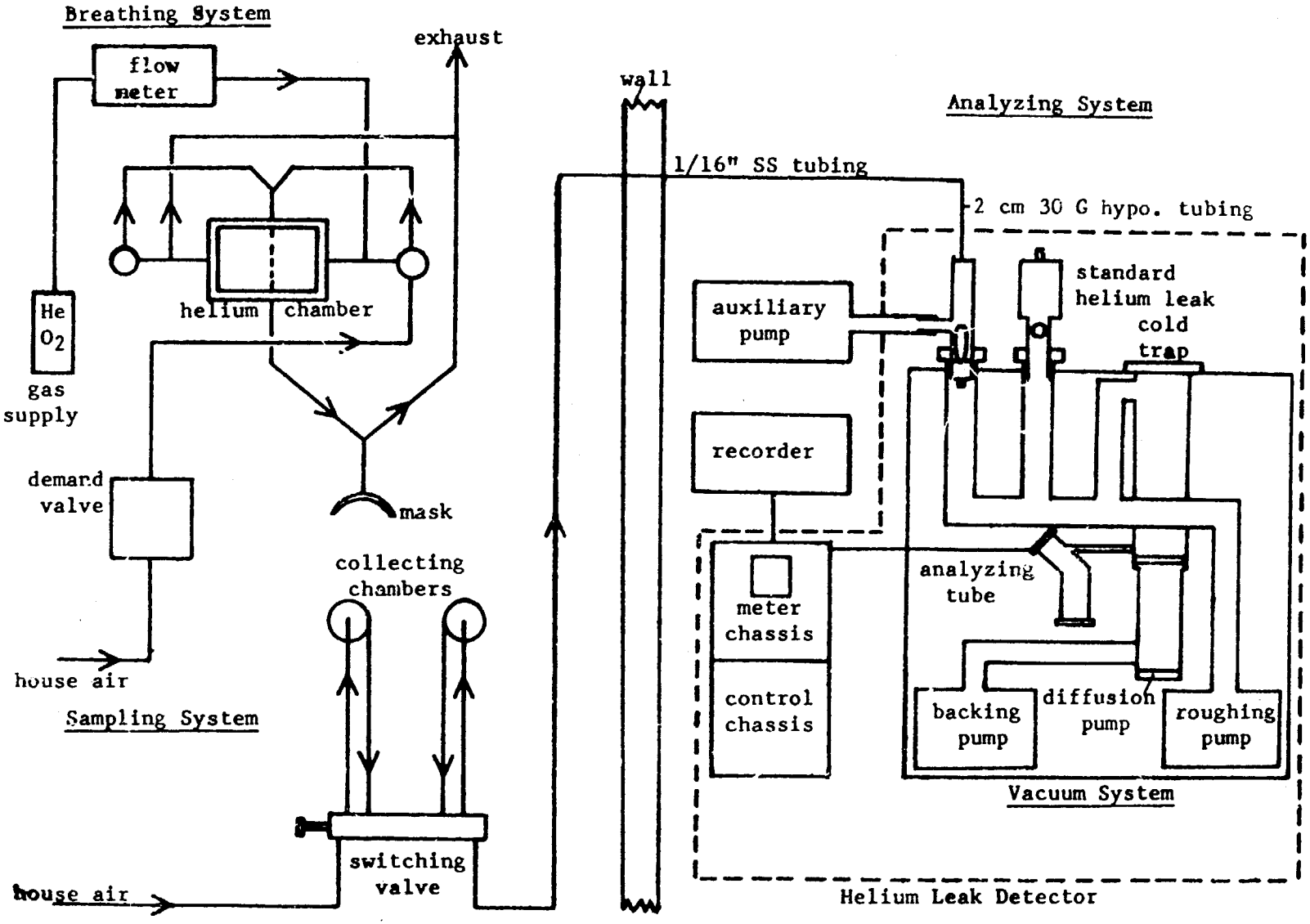


Figure III-5 System for continuous helium analysis.

edges of the top plate to serve as attachments for a 5/8" woven elastic band used to hold the chamber firmly on the skin of the forearm. The luer-tapered apertures in the top of the chamber were fitted with nylon needle adapters connected to 1/8" I.D. Tygon tubing. Air flow through the chamber was maintained at 1 liter per minute by manual adjustment of a reducing valve and monitored with a rotameter type flow meter. The detection of water vapor as variations in relative humidity was essentially the same as that described by Bullard (33) modified to include separate thermocouples within the enclosure provided for each of seven narrow-range Hydrodynamics humidity sensors. The perforated rear cover of a Model No. 4-5176 Hygrodynamics Multiple Mounting was removed, the entire unit was imbedded in the 4-inch styrafoam insulation of the constant temperature room and each of the eight sensors was enclosed in the cut-off barrel of a 30 cc, plastic, disposable syringe with the intact syringe tip projecting through a hole and accessible from the interior of the temperature-controlled room for coupling with the tube carrying effluent air from the arm chamber. The sensor having the range appropriate to the conditions of the experiment was determined empirically. A selector switch on the outer cover of the Multiple Mounting connected the appropriate sensor through a Model 5-3000 Indicator to the Grass polygraph. A separate selector switch was used to connect the thermocouple within the enclosure of the sensor being used to a 5P1 Grass preamplifier for recording on the Polygraph.

All temperatures measured by means of thermistors were recorded on a single channel of the Polygraph by using a selector switch to connect any of six thermistors with the recorder through a YSI Model 42 Telethermometer. The calibrated heat-flow disks were connected directly to 5P1 Low Level preamplifiers of the Polygraph.

2. Helium analysis by means of a mass spectrometer type helium leak tester. 1968-70

a. Breathing system. Figure III-5 shows in schematic form the breathing system which supplied air and either fixed volumes of helium - oxygen mixture or a continuous supply of helium - oxygen to the subject by way of a Bennet face mask. Two "J" valves limited rebreathing to the volume of gas contained in about one foot of corrugated tubing and the space within the mask. Air was supplied from the house air system. The illustration shows a "helium chamber" of 1-liter capacity, which was used to administer helium - oxygen in fixed doses. Later development of our breathing system included a separate demand valve directly connected to the size "G" helium - oxygen cylinder. A three-way valve was used to deliver either continuous air or helium - oxygen mixture through the one-way breathing system.

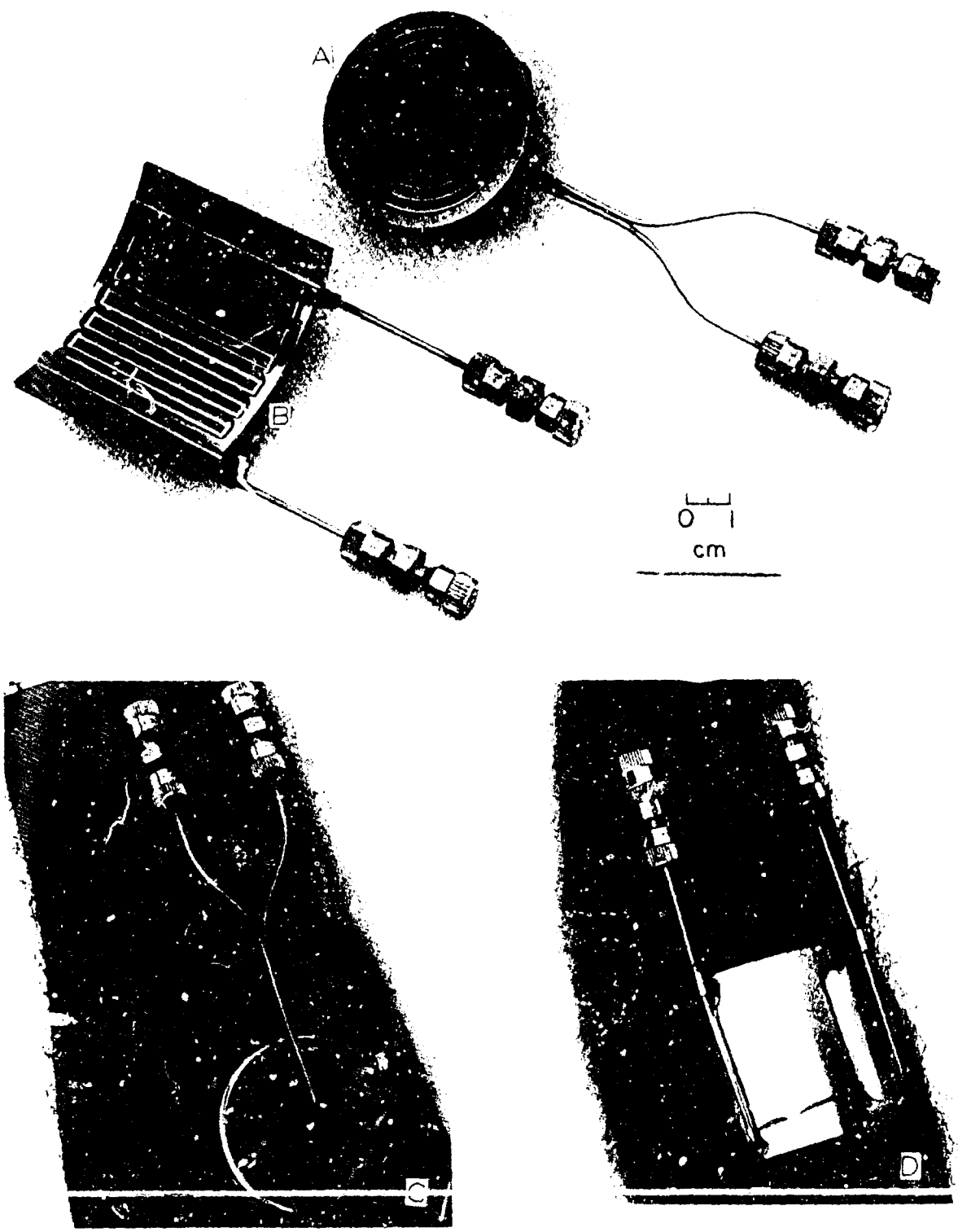


Figure III-6 Helium sampling chambers.

All exhaled air was vented through the top of the controlled-temperature room through a vacuum cleaner hose attached to a low-capacity blower. This arrangement was found to be necessary in order to prevent large accumulations of helium in the air of the room.

b. Sampling system. The system shown in figure III-5 was devised to permit alternate sampling from each of two chambers secured to the skin of the subject. The sampling chambers shown in figure III-6 are designed to conduct a one-way flow of air over 13.2 cm^2 of skin without significant mixing errors. The chamber shown in figure III-6 (A) is similar to that used by Adamczyk and his associates (44). It is satisfactory for skin areas flat enough to permit leak-free sealing to the skin without undue pressure. We designed the chamber shown in figure III-6 (B) to sample curved surfaces of comparable area. It is easily attached to curved surfaces such as the upper surface of the forearm when the hand is in the pronated position. Both types of chamber were attached to the skin by means of a gasket cut from surgical double-faced adhesive film (3M Stomaseal (R).

The connectors shown in the illustration are 1/16" unions which make a vacuum-tight connection by means of "O" rings compressed about the 1/16" stainless steel tubing soldered to the chambers and the one-meter lengths of tubing used to connect the chambers to the switching valve. The switching valve was a Loenco Model 206-6 designed for use in gas chromatography systems to switch the flow of carrier gas from one column to another. In the original application it served to switch dried house air at 1.5 cm water pressure from one chamber to another and, in the same motion, to switch the effluent from that chamber to the line joining the sampling port of the helium leak tester. The sample line, also of 1/16" stainless steel tubing passed through the rear wall of the controlled temperature room to an adjacent room where the auxiliary pump, leak tester and recorder were located. Although this arrangement added a few milliseconds to the response-time, it was necessary to avoid increasing the heat load and noise in the room with the subjects.

A more recent modification of the connections and the addition of a second switching valve has permitted continuous flushing of the chamber that is not being sampled and a provision for by-passing both chambers so that dried house air can be delivered to the leak tester for zero check. Air pressure within the collection chamber was adjusted to a few mm above ambient pressure.

After the sample line passed through the wall of the controlled temperature room, it was connected through a bellows type, very-fine-metering valve into a rotameter. The gas was then delivered from the rotameter into the sample introduction

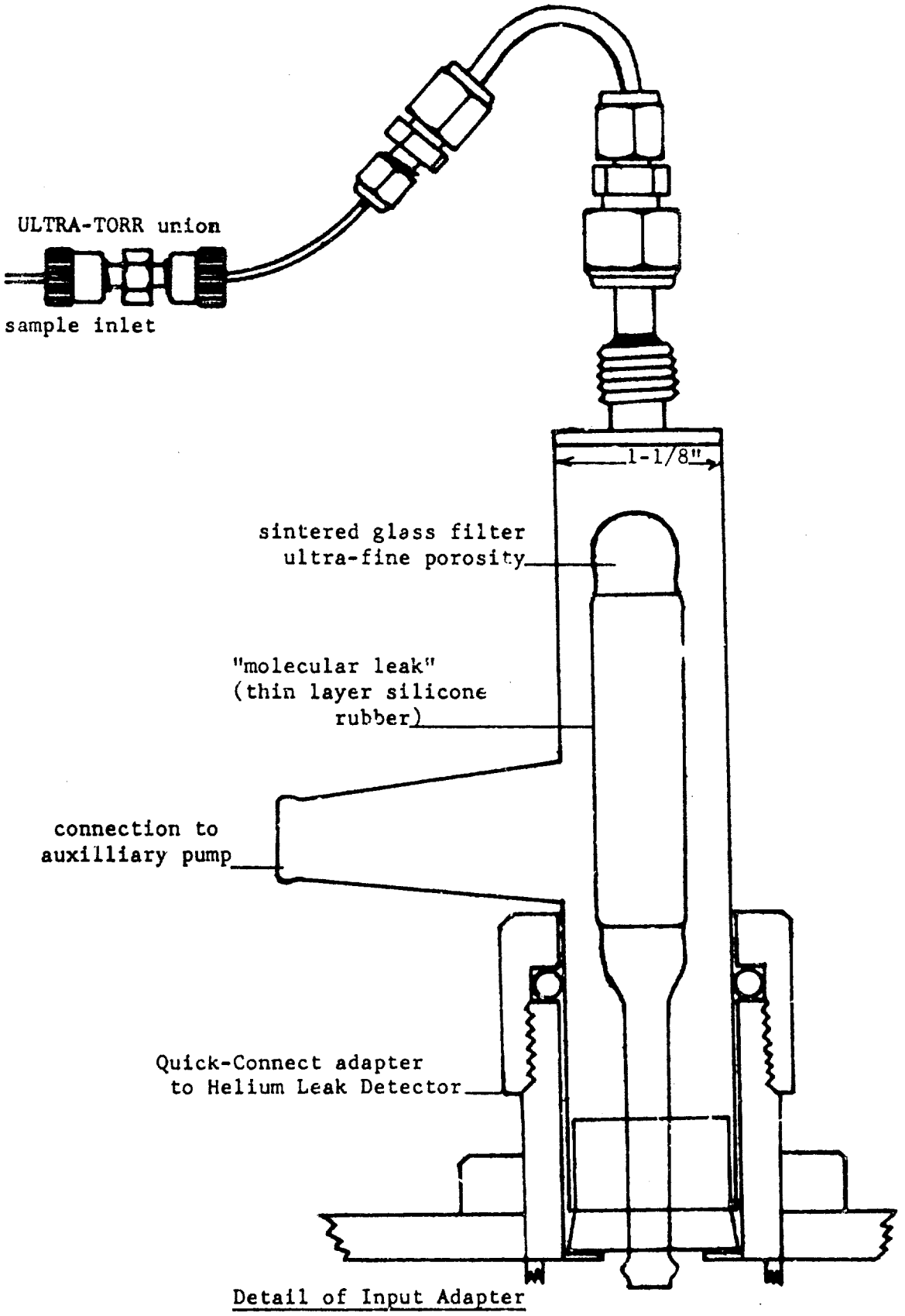


Figure III-7 Sample introducing system.

system. An auxiliary vacuum pump, connected to the side tubulature of the gas-introducing system, furnished the principal pressure gradient for sample flow. A second bellows metering valve in the line from the auxiliary vacuum pump completed the system for adjusting both the operating pressure in the leak tester and the flow rate of the gas being sampled from the arm chamber.

c. Sample Introduction System. The analysis of helium in a helium leak tester, as in the mass spectrograph, depends upon the ionization of gas atoms in a vacuum. The optimum pressure for gaseous samples in most ion sources is about 10^{-4} mmHg. In order to keep gas over the skin areas being tested close to atmospheric pressure, some type of constriction is necessary between the sampling chamber on the skin and the ionization chamber of the spectrometer tube (analyzing tube in figure III-5). Our first attempt to meet these conditions was similar to that employed by Adamczyk and his associates (44). We used about two meters of 27-gauge hypodermic tubing as the input from the gas collection chamber on the skin to the leak tester. The pumping speed of the leak tester plus the auxiliary pump was unable to maintain the required vacuum. By nearly complete closure of the internal throttling valve of the leak tester we were able to maintain leak tester pressure at 10^{-4} torr. Measurements of helium could be made but only at the sacrifice of about 75 per cent of the potential sensitivity of the system. Figure III-7 illustrates a second method of restricting gas flow into the vacuum system of the leak tester. The restrictor was made by coating a sintered glass filter tube with a thin layer of silicone rubber (RTV 108 translucent adhesive dissolved in toluene) and baking for 2 hours at 250°C. The coated filter was then pushed through a #8 one-hole rubber stopper. With the stopper pushed firmly into the 1-1/8" brass T adapter, the adapter was inserted into the sampling port of the leak tester until the rubber stopper rested on the internal flange of the inlet tube. The internal surface of the sintered glass filter was exposed to the vacuum of the leak tester; the outer surface coated with silicone rubber was exposed to the gas stream from the sampling chamber on the subject's skin. The restrictor limited the penetration of gas into the vacuum system of the leak tester well enough to permit full opening of the internal throttling valve and a sustained internal pressure of $10^{-4.5}$ torr. Silicone rubber is relatively permeable to fixed gases. The dispersion of helium at the analyzing tube of the leak tester indicated that the restrictor had some of the characteristics of a molecular leak. Our equipment does not permit a rigorous characterization of the properties of the leak with respect to gases present other than helum; the term molecular leak is used in quotation marks because of this uncertainty. Empirical calibration with mixtures from 0 to 150 µl/liter of helium in air showed a linear

SAMPLE SPLITTER

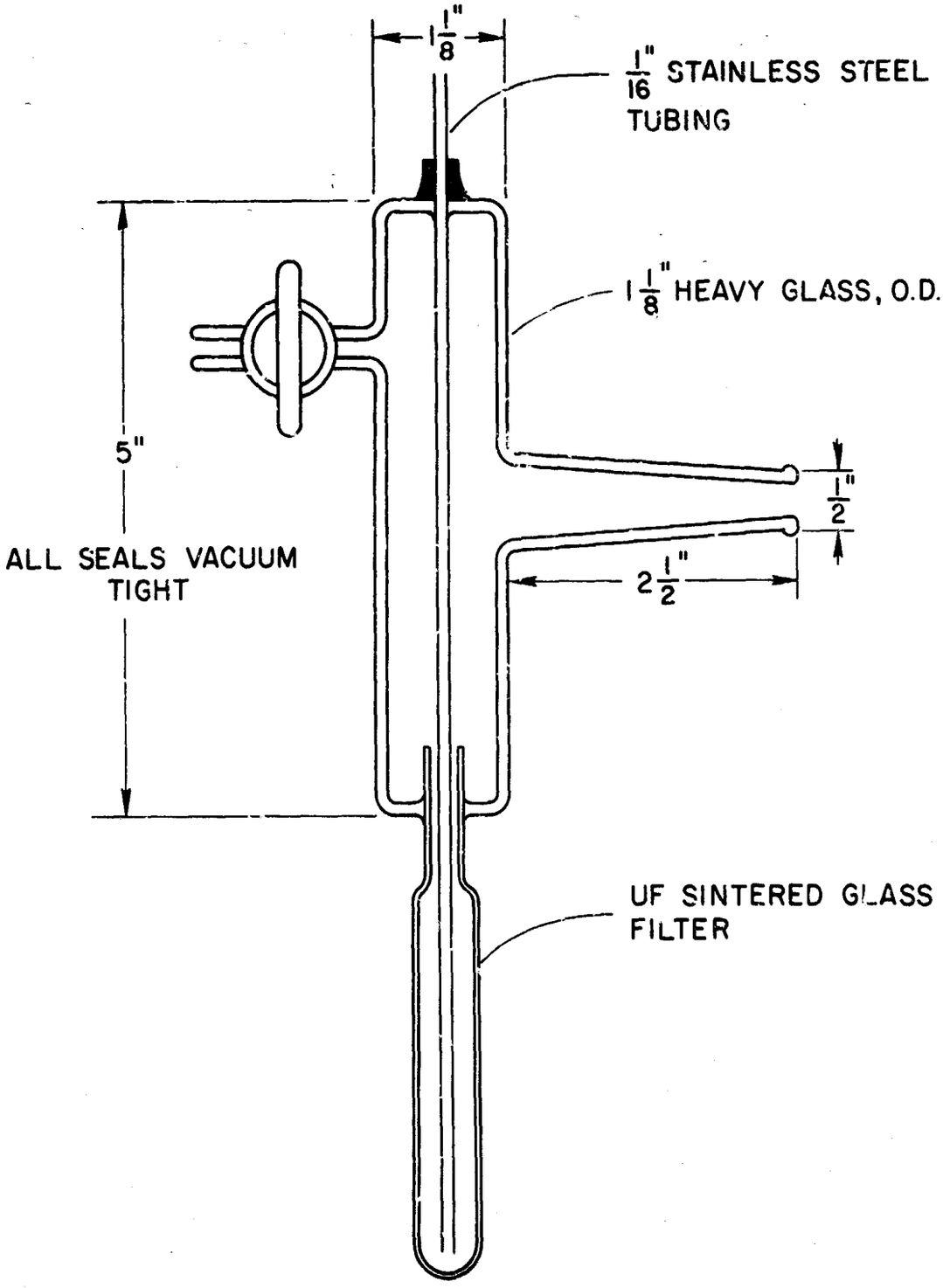


Figure III-8 All-glass sample introducing system.

relationship of recorder output to helium concentration of the introduced sample. The partial pressure of helium at the detector was thus linearly related to the partial pressure of helium in the original gas sample.

Figure III-8 shows a more recent (1969) version of the sample-introduction system. It is a fused glass chamber with a 1/16" stainless steel tube sealed to the inlet for the sample. The steel tube was sealed to the glass envelope with a special vacuum-tight epoxy cement.

d. Calculation procedure.

The bellows valves and rotameter installed in the sample introduction system (see Appendix III, p.) have made it possible to set the flow rate of sampling gas through the collection chamber. By analysis of known concentrations of helium in air at known sample flow rates, we have derived "splitting factors" which express the ratio of actual helium presented by the sampling stream to the indicated amount of helium shown by the output of the leak rate meter. In calculating helium leak rates from the skin, the indicated leak rate is devided by the skin area sampled to give the leak rate in atmosphere (atm) cc of helium/cm^2/ sec. This rate is then multiplied by the "splitting factor" characterizing the sampling flow rate used to obtain the true helium leak rate from the skin. Periodic internal calibration of the helium leak tester is accomplished by means of a calibrated standard helium leak. In our instrument, the calibrated standard leak furnished 8.7×10^{-8} atm/cc/sec.

Sample calculation: (Skin area sampled = 13.6 cm^2).
(Splitting factor = 310.7.)

$$\text{Helium Leak Rate} = \frac{\text{Std. Leak Rate} \times \text{Scale Factor} \times \text{recorder divisions}}{\text{Scale factor of sample} \times \text{recorder divisions of sample}}$$

$$\text{Uncorrected HLR} = \frac{8.7 \times 10^{-8} \times 10 \times 15 \text{ div.}}{10 \times 7.5 \text{ div.}}$$

$$= \frac{1{,}305 \times 10^{-8}}{75}$$

$$\text{HLR (corrected for area)} = 17.4 \times 10^{-8} \text{atm cc/sec}/13.6 \text{ cm}^2$$

$$= \frac{17.4 \times 10^{-8} \text{atm cc/sec}}{13.6 \text{ cm}^2} = 0.1279 \times 10^{-8} \frac{\text{atm cc}}{\text{cm}^2/\text{sec}}$$

HLR (corrected for splitting factor)

$$= 0.1279 \times 10^{-8} \times 310.7 = 5.21 \times 10^{-8} \text{ atm/cc/cm}^2\text{/sec}$$

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13. ABSTRACT

Studies carried out under this contract have been directed toward (a) an examination of the nature of blood flow distribution within the skin and (b) a study of participation of superficial dermal capillaries in major changes of skin blood flow rate. This report consists of four Parts. Three Appendixes contain technical details of equipment construction and development. Part 1 contains a statement of objectives and a section on background. Part 2 is a summary of preliminary work including initial hypotheses and development of methods. Results of experiments testing the feasibility of using clearance rate of radioactive isotopes to measure effective skin blood flow showed that the method was too slow to display rapid changes in blood flow and potentially dangerous for repeated use in the same subject. Existing methods of measuring total forearm blood flow were not suitable for our needs. The development of temperature-stable forearm blood flow gauges produced two potentially useful designs. A small, light-weight gauge that was based on changes of capacitance with changes of arm volume was little affected by temperature, but solution of cable and calibration problems would have required an additional year's work. The device finally developed was a mercury-in-silastic rubber gauge modified from the original design by Whitney. The improved gauge can be calibrated electrically *in situ* by means of part of the control circuit. The relationship of change in arm volume after venous occlusion to voltage output is independent of temperature.

Part 2 also describes preliminary work with helium transfer through the skin as an index of effective skin blood flow. Helium was collected from plastic capsules cemented to forearm skin. When the subject breathed a mixture of 80% helium and 20% oxygen, a collection period of 10 minutes yielded sufficient helium from about 20 cm^2 of skin to permit accurate measurement on 1 ml samples by gas chromatography. Data are present-

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ed on simultaneous measurements of skin temperature, total forearm blood flow, sweat rate and helium flux through the skin under various conditions. Effects of heat, cold, posture and exercise, and shunting of blood flow through arteriovenous anastomoses are described.

Part 3 presents the development of equipment and procedures for continuous analysis of helium at a sensitivity about six orders of magnitude greater than that of the gas chromatograph. The high sensitivity was made possible by sweeping a stream of air over about 13 cm^2 of skin and delivering about 1/300 of the 0.5 ml/sec flow to a mass spectrometer-type helium leak tester. Additional studies were carried out on effects of upright posture on skin blood flow distribution. The change from reclining to upright posture was simulated by application of short periods of lower-body negative pressure (LBNP) to reclining subjects. During the redistributions of peripheral resistance evoked by the decreased cardiac output during LBNP, forearm blood flow decreased. The small changes in skin temperature and the slight decreases in helium leak rate suggested that the resistance vessels of the skin were engaged to a relatively small extent in the reflex vasoconstriction.

The relationships of minute volume and distribution of skin blood flow to processes of heat loss were studied in detail. During general body heating of resting subjects, helium leak rate increased in proportion to rising skin temperature. Direct participation of sweat gland activity in the increased rates of helium transfer was tested by adding 7% CO_2 to the breathing mixture. Inhalation of carbon dioxide evokes sweating when core and skin temperatures were maintained below sweating threshold. Although sweating was produced in small amounts by CO_2 inhalation, the changes in forearm blood flow and helium leak rate were inconstant if skin temperatures were kept below 35.5°C. When ion transfer of atropine into skin of one forearm was used to block sweat secretion in heated subjects, the blood flow in the atropinized arm rose above that in the control arm. Helium leak rates were also higher in the atropinized arm than in the control arm in spite of the absence of sweating in the blocked arm and the vigorous sweating in the control arm. Direct heating of one arm of a subject exposed to comfortably cool ambient temperature produced extremely high helium leak rates and large increases of forearm blood flow. Isoproterenol administered by ion transfer to one arm produced relaxation of resistance vessels in treated skin, but there was no evidence that precapillary sphincters of superficial dermal capillaries responded to the vasodilator. The differences in helium leak rate and forearm blood flow were similar in direction to those produced by local heating but much smaller in magnitude.

Part 4 contains a general discussion of results, including comparison of our data on the relation of helium leak rate to skin temperature and calculations of skin blood flow required for maintaining core and skin temperatures constant. Our data are also compared to the findings of others. Conclusions based upon our findings are: 1. The rate of leakage of helium through skin is directly proportional to the capillary area available for exchange and inversely proportional to the distance from the capillary surface to the skin surface. When the number of open capillaries increases, the rate of perfusion increases. Therefore the helium leak rate may be used as an index of blood flow distribution in the skin. 2. If some of the skin blood flow is shunted through channels other than the superficial capillaries, the rate of helium leakage is small relative to the total skin blood flow. 3. The distribution of blood flow in fore-

arm skin is very little affected by barostatic reflexes such as those involved in redistributions of peripheral resistance in response to changing from reclining to upright posture. 4. Circulation of increased amounts of blood in vessels supplying the coiled portion of sweat glands and sweat gland ducts provides a functional blood shunt during responses to general body heating. Little decrease of resistance to flow occurs in the superficial capillary bed when the skin is cooled by evaporation of sweat. 5. Interference with the evaporation of sweat in subjects responding to high ambient temperature or direct application of heat to the skin increases the number of open capillaries as indicated by increased helium leak rate, erythema, and direct counts of visible capillary loops. 6. The precapillary sphincters of the most superficial distribution in the skin do not dilate in response to any of the various changes of nerve activity involved in the heat-loss response. They are controlled by autoregulation in response to a change in heat or to some feature of the metabolic response to a change in heat.

Recommendations included in the report are: 1. Studies should be carried out to determine the possible usefulness of measurements of helium leak rate to plastic surgeons interested in the rate of revascularization of skin grafts. 2. Studies should be carried out to determine the possible role of dilatation of superficial skin vessels in heat exhaustion and heat stroke. Both effective blood volume and lowered total peripheral resistance may be involved. 3. Studies should be carried out to determine how circulatory stress is related to the rate of acclimatization to heat. Heat acclimatization might occur more rapidly and more consistently in subjects in whom the heat stress included judiciously limited increases in skin temperature.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
skin blood flow						
helium excretion by skin						
skin capillary flow						
gas loss through skin						